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(54) Title: COMPOSITIONS AND METHODS FOR TREATING NEUROGENIC DISORDERS OF THE PELVIC FLOOR

(57) Abstract: Provided herein are methods for the treatment of bladder dysfunction, including detrusor hyperreflexia and detrusor external sphincter dyssynergia, fecal incontinence, and/or sexual dysfunction in an individual via the use of stably expressed light-responsive opsin proteins capable of selective hyperpolarization or depolarization of the neural cells that innervate the muscles responsible for physiologic functioning of urinary bladder, external urinary sphincter, external anal sphincter, and the male and female genitalia.



COMPOSITIONS AND METHODS FOR TREATING NEUROGENIC DISORDERS OF THE PELVIC FLOOR

CROSS-REFERENCE

[0001] This application claims the benefit of U.S. Provisional Patent Application No. 61/601,298, filed February 21, 2012, which application is incorporated herein by reference in its entirety.

TECHNICAL FIELD

[0002] This application pertains to methods for treating neurogenic disorders of the pelvic floor including bladder dysfunction, fecal incontinence, and sexual dysfunction in an individual, via the use of stably expressed light-responsive opsin proteins capable of selectively altering the membrane potential of the neural cells that innervate the muscles and organs responsible for urinary, rectal, and sexual function.

INTRODUCTION

[0003] “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. The hallmark of optogenetics is the introduction of fast light-responsive ion channel and/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 (*See, e.g.* U.S. Patent Application Publication Nos. 2007/0054319, 2009/0093403, and 2010/0145418 as well as International Patent Application Publication Nos. WO 2009/131837 and WO 2007/024391). 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 *in vivo*, including the interactions between skeletal and smooth muscles with the neurons responsible for their innervation.

[0004] Urinary incontinence, the inability to maintain voluntary control of micturition, is a condition that affects millions of men and women worldwide. The control of urination is a complex physiological process including neural reflex pathways, some with and some without central nervous system control, smooth and voluntary muscles, as well as hormonal effects. (*See* review by DeGroat, 1997, *Urology* 50[Supp6A]:36-52). A large subset of urinary incontinence is at least partly neurogenic. The clinical term “overactive bladder” is used generally to denote any form of incontinence characterized by increased frequency of micturition or desire to void, whether complete or episodic, and where loss of voluntary control ranges from partial to total. “Urge incontinence” is the involuntary loss of urine associated with an abrupt and powerful desire to void. Urge incontinence is often associated with the urodynamic finding of involuntary (uninhibited)

contractions of the detrusor muscle, which provides the primary force in expelling urine from the bladder. A large subset of patients with uninhibited detrusor have some sort of neurologic impairment, in which case the clinical term is “detrusor hyperreflexia” (DH). Common neurologic disorders associated with detrusor hyperreflexia (DH) are Parkinson's disease, stroke, diabetes, multiple sclerosis (MS), and peripheral neuropathy. Additionally, individuals who suffer a traumatic injury to the spinal cord commonly experience symptoms associated with DH.

[0005] The muscles of the external urinary sphincter may also be affected by spinal cord injuries, resulting in a condition known as “dyssynergia.” Dyssynergia involves an inability of urinary sphincter muscles to relax when the bladder contracts, including active contraction in response to bladder voiding, preventing urine from flowing through the urethra and resulting in the incomplete emptying of the bladder and “reflux” of urine into the kidneys.

[0006] Some treatments for detrusor hyperreflexia and external urinary sphincter dyssynergia rely on electrical neurostimulation. This treatment modality depends on the use of electrodes positioned adjacent to the nerve/muscle to be stimulated. Activation of the electrode with an electrical pulse excites the adjacent nerve leading to the contraction of muscles innervated by that nerve. However, the electrode is non-selective and will stimulate every tissue and cell type that falls within its electrical field. Thus, current neurostimulatory methods and devices cannot act locally with cell-type specificity to regulate the muscles and the nerves responsible for symptoms associated with detrusor hyperreflexia and external urinary sphincter dyssynergia.

[0007] Sacral anterior root stimulation (SARS, Finetech Medical Limited) is a neurostimulatory device used to restore bladder function in patients who have sustained spinal cord injuries. SARS requires a sacral root rhizotomy to prevent DH/DSD, resulting in loss of sexual function. Additionally, sacral root rhizotomy further damages the afferent pathways of the lower urinary tract, leading to bladder areflexia or the loss of bladder contraction. As a result, post-surgical electrical stimulation is needed to activate the detrusor muscle. However, at the same time, the electrode also activates the external urinary sphincter muscle because the nerves innervating the sphincter are larger than those of the detrusor and, therefore, are recruited first. Because the striated muscle of the sphincter relaxes more rapidly than the smooth muscle of the detrusor, an individual who has undergone SARS experiences post-stimulus voiding for a brief period of time. However, in addition to the aforementioned loss of sexual function, SARS often results in very high bladder pressure that can cause near-term vesicoureteral reflux and has been associated, long-term, with renal failure.

[0008] Other current treatments for detrusor hyperreflexia and external urinary sphincter dyssynergia include permanent or intermittent self-catheterization combined with anti-muscarinic agents (such as oxybutynin or tolterodine), use of alpha-blockers, injection of the external urinary sphincter with Botulinum Toxin (such as, Botox® (Allergan) or Dysport® (Ipsen)), balloon dilation of the external urinary sphincter, and use of urethral stents. However, all of these treatments suffer from considerable drawbacks, including the need for frequent repeat treatments (in the case of

intermittent catheterization and Botulinum Toxin administration), increased urinary tract infections (catheterization, stents, balloon dilation), increased incidence of septicemia (balloon dilation), and increased susceptibility to squamous-cell carcinoma (catheterization).

[0009] Another pelvic floor disorder, fecal incontinence, is a condition whereby fecal material is involuntarily excreted or leaked due to decreased bowel control. Fecal incontinence of varying degrees is thought to be a result of any number of factors, including dysfunction of or damage to the anal sphincters, dysfunction of the pelvic floor, or decreased compliance in the rectum. The neural supply to the anorectal region is both somatic and autonomic. The superficial perineal nerve (branch of pudendal nerve) provides sensory fibers to the perineum as well as the anal canal mucosa. The external anal sphincter receives its motor supply from the inferior rectal nerve (a.k.a. inferior hemorrhoidal nerve, a branch of the pudendal nerve). Like treatments for urinary dysfunction, some treatments for fecal incontinence rely on electrical stimulation of the nerves that control the muscles of the anal sphincter and attempt to restore an individual's continence. Other surgical treatments, such as colostomy, are also common. However, all of these methods suffer from considerable practical drawbacks and complications.

[0010] Sexual dysfunction comprises a broad range of maladies, including erectile dysfunction, orgasmic dysfunction, premature ejaculation and lack of lubrication. Sexual dysfunctions plague both women and men, and may be life-long or acquired. Sexual dysfunction has a number of causes, both physiological and psychological, and in many patients the disorder may be multifactorial. The causes include several that are essentially neurologic in origin. For example, damage to the pathways used by the autonomic nervous system to innervate the penis and clitoris may interrupt sexual arousal initiated by the central nervous system. Lesions (*e.g.*, injury, infection, or disease) of the somatic nervous pathways (*i.e.*, any of the nerves associated with sensation or motion) may impair reflexogenic sexual function (*i.e.*, involuntary, instinctive physiological response to a stimulus) and may interrupt tactile sensation needed to maintain sexual arousal. Additionally, spinal cord lesions may produce varying degrees of sexual dysfunction depending on the location and severity of the lesions.

[0011] Currently, there is no good long acting, cost effective, or clinically meaningful therapy that precisely targets the neural cells and associated muscles/organs responsible for these defects with the potential to bring about more physiologically normal micturition, bowel control, and sexual function in individuals with symptoms associated with neurogenic disorders of the pelvic floor.

[0012] 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.

SUMMARY

[0013] Provided herein are methods for the treatment of bladder dysfunction, fecal incontinence, and sexual dysfunction in an individual via the use of stably expressed light-responsive opsin proteins capable of hyperpolarizing or depolarizing the neurons that innervate the muscles of the bladder, the

neurons that innervate the external urinary sphincter, the neurons that innervate the external anal sphincter, the neurons that innervate the muscles responsible for the contraction of the rectum, and/or the neurons that innervate the genitalia, wherein the hyperpolarization or depolarization-induced synaptic depletion of the neurons induces the relaxation of the muscle innervated by the neurons, and depolarization of the neurons induces the contraction of the muscle innervated by the neurons. In some embodiments, contraction of the detrusor muscle and relaxation of the external urinary sphincter to permit urination may be induced by the activation of one or more of the light-responsive opsin proteins expressed in the neurons. In some embodiments, relaxation of the detrusor muscle and contraction of the external urinary sphincter to allow urine storage may be induced by the activation of one or more of the light-responsive opsin proteins expressed in the neurons. In some embodiments contraction of the external anal sphincter to permit storage of stool may be induced by the activation of one or more light-responsive opsin proteins expressed in the neurons. In some embodiments, relaxation of the external anal sphincter to permit defecation may be induced by the activation of one or more of the light-responsive opsin proteins expressed in the neurons. Furthermore, defecation may be additionally facilitated by simultaneous contraction of the muscles responsible for the contraction of the rectum induced by the activation of one or more of the light-responsive opsin proteins expressed in the neurons. In some embodiments, sexual dysfunction may be treated by increasing the depolarization of the neurons responsible for the innervation of the muscles and organs of the genitalia.

[0014] Accordingly, in some aspects, provided herein is a method for treating a bladder dysfunction in an individual in need thereof, the method comprising: administering an effective amount of a polynucleotide comprising a nucleotide sequence encoding a light-responsive opsin protein to the individual, wherein the opsin protein is expressed on the plasma membrane of a population of neurons responsible for the innervation of the detrusor muscle and/or a population of neurons responsible for the innervation of the external urinary sphincter muscle in the individual, wherein the opsin protein induces hyperpolarization of the neurons expressing the opsin protein in response to light, whereby the hyperpolarization of the neurons responsible for the innervation of the detrusor muscle permits storage of urine in the bladder and the hyperpolarization of the neurons responsible for the innervation of the external urinary sphincter muscle permits voiding urine from the bladder, wherein the polynucleotide comprises a nucleotide sequence encoding a light-responsive opsin protein comprising a sequence at least 95% identical to the sequence shown in SEQ ID NO:1, an ER export signal, and a membrane trafficking signal.

[0015] In some aspects, provided herein is a method for treating a bladder dysfunction in an individual in need thereof, the method comprising: administering an effective amount of a polynucleotide comprising a nucleotide sequence encoding a light-responsive opsin protein to the individual, wherein the opsin protein is expressed on the plasma membrane of a population of neurons responsible for the innervation of the detrusor muscle and/or a population of neurons

responsible for the innervation of the external urinary sphincter muscle in the individual, wherein the opsin protein induces hyperpolarization of the neurons expressing the opsin protein in response to light, whereby the hyperpolarization of the neurons responsible for the innervation of the detrusor muscle permits storage of urine in the bladder and the hyperpolarization of the neurons responsible for the innervation of the external urinary sphincter muscle permits voiding urine from the bladder, wherein the polynucleotide comprises a nucleotide sequence encoding a light-responsive opsin protein comprising a sequence at least 95% identical to the sequence shown in SEQ ID NO:23, an ER export signal, and a membrane trafficking signal.

[0016] In some aspects, provided herein is a method for treating a bladder dysfunction in an individual in need thereof, the method comprising: administering an effective amount of a polynucleotide comprising a nucleotide sequence encoding a light-responsive opsin protein to the individual, wherein the opsin protein is expressed on the plasma membrane of a population of neurons responsible for the innervation of the detrusor muscle and/or a population of neurons responsible for the innervation of the external urinary sphincter muscle in the individual, wherein the opsin protein induces hyperpolarization of the neurons expressing the opsin protein in response to light, whereby the hyperpolarization of the neurons responsible for the innervation of the detrusor muscle permits storage of urine in the bladder and the hyperpolarization of the neurons responsible for the innervation of the external urinary sphincter muscle permits voiding urine from the bladder, wherein the polynucleotide comprises a nucleotide sequence encoding a light-responsive opsin protein comprising a sequence at least 95% identical to the sequence shown in SEQ ID NO:4, an ER export signal, and a membrane trafficking signal.

[0017] In other aspects, provided herein is a method for treating a bladder dysfunction in an individual in need thereof, the method comprising: administering an effective amount of a polynucleotide comprising a nucleotide sequence encoding a light-responsive opsin protein to the individual, wherein the opsin protein is expressed on the plasma membrane of a population of neurons responsible for the innervation of the detrusor muscle and/or a population of neurons responsible for the innervation of the external urinary sphincter muscle in the individual, wherein the opsin protein is capable of inducing depolarization-induced synaptic depletion of the neurons expressing the opsin protein in response to light, whereby the depolarization-induced synaptic depletion of the neurons responsible for the innervation of the detrusor muscle permits storage of urine in the bladder and the depolarization-induced synaptic depletion of the neurons responsible for the innervation of the external urinary sphincter muscle permits voiding urine from the bladder, wherein the polynucleotide comprises a nucleotide sequence encoding a light-responsive opsin protein comprising a sequence at least 95% identical to the sequence selected from the group consisting of SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, and SEQ ID NO:11.

[0018] In some aspects, also provided herein is a method for treating a bladder dysfunction in an individual in need thereof, the method comprising: administering an effective amount of a polynucleotide comprising a nucleotide sequence encoding a light-responsive opsin protein to the individual, wherein the opsin protein is expressed on the plasma membrane of a population of neurons responsible for the innervation of the detrusor muscle and/or a population of neurons responsible for the innervation of the external urinary sphincter muscle in the individual, wherein the opsin protein is capable of inducing depolarization of the neurons, whereby the depolarization of the neurons responsible for the innervation of the detrusor muscle permits voiding urine from the bladder and the depolarization of the neurons responsible for the innervation of the external urinary sphincter muscle permits storage of urine in the bladder, wherein the polynucleotide comprises a nucleotide sequence encoding a light-responsive opsin protein comprising a sequence at least 95% identical to the sequence selected from the group consisting of SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, and SEQ ID NO:11.

[0019] In other aspects, provided herein is a method for treating fecal incontinence in an individual in need thereof, the method comprising: administering an effective amount of a polynucleotide comprising a nucleotide sequence encoding a light-responsive opsin protein to the individual, wherein the opsin protein is expressed on the plasma membrane of a population of neurons responsible for the innervation of the external anal sphincter muscle in the individual, wherein the opsin protein induces hyperpolarization of the neurons expressing the opsin protein in response to light, whereby the hyperpolarization of the neurons responsible for the innervation of the external anal sphincter muscle permits defecation, wherein the polynucleotide comprises a nucleotide sequence encoding a light-responsive opsin protein comprising a sequence at least 95% identical to the sequence shown in SEQ ID NO:1, an ER export signal, and a membrane trafficking signal.

[0020] In other aspects, provided herein is a method for treating fecal incontinence in an individual in need thereof, the method comprising: administering an effective amount of a polynucleotide comprising a nucleotide sequence encoding a light-responsive opsin protein to the individual, wherein the opsin protein is expressed on the plasma membrane of a population of neurons responsible for the innervation of the external anal sphincter muscle in the individual, wherein the opsin protein induces hyperpolarization of the neurons expressing the opsin protein in response to light, whereby the hyperpolarization of the neurons responsible for the innervation of the external anal sphincter muscle permits defecation, wherein the polynucleotide comprises a nucleotide sequence encoding a light-responsive opsin protein comprising a sequence at least 95% identical to the sequence shown in SEQ ID NO:4, an ER export signal, and a membrane trafficking signal.

[0021] In other aspects, provided herein is a method for treating fecal incontinence in an individual in need thereof, the method comprising: administering an effective amount of a polynucleotide comprising a nucleotide sequence encoding a light-responsive opsin protein to the individual, wherein the opsin protein is expressed on the plasma membrane of a population of neurons

responsible for the innervation of the external anal sphincter muscle in the individual, wherein the opsin protein induces hyperpolarization of the neurons expressing the opsin protein in response to light, whereby the hyperpolarization of the neurons responsible for the innervation of the external anal sphincter muscle permits defecation, wherein the polynucleotide comprises a nucleotide sequence encoding a light-responsive opsin protein comprising a sequence at least 95% identical to the sequence shown in SEQ ID NO:23, an ER export signal, and a membrane trafficking signal.

[0022] In yet other aspects, provided herein is a method for treating fecal incontinence in an individual in need thereof, the method comprising: administering an effective amount of a polynucleotide comprising a nucleotide sequence encoding a light-responsive opsin protein to the individual, wherein the opsin protein is expressed on the plasma membrane of a population of neurons responsible for the innervation of the external anal sphincter muscle in the individual, wherein the opsin protein is capable of inducing depolarization-induced synaptic depletion of the neurons expressing the opsin protein in response to light, whereby the depolarization-induced synaptic depletion of the neurons responsible for the innervation of the external anal sphincter muscle permits defecation, wherein the polynucleotide comprises a nucleotide sequence encoding a light-responsive opsin protein comprising a sequence at least 95% identical to the sequence selected from the group consisting of SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, and SEQ ID NO:11.

[0023] In another aspect, provided herein is a method for treating fecal incontinence in an individual in need thereof, the method comprising: administering an effective amount of a polynucleotide comprising a nucleotide sequence encoding a light-responsive opsin protein to the individual, wherein the opsin protein is expressed on the plasma membrane of a population of neurons responsible for the innervation of the external anal sphincter muscle in the individual, wherein the opsin protein is capable of inducing depolarization of the neurons, whereby the depolarization of the neurons responsible for the innervation of the external anal sphincter muscle permits storage of stool in the rectum, wherein the polynucleotide comprises a nucleotide sequence encoding a light-responsive opsin protein comprising a sequence at least 95% identical to the sequence selected from the group consisting of SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, and SEQ ID NO:11.

[0024] In other aspects, provided herein is a method for treating sexual dysfunction in an individual in need thereof, the method comprising: administering an effective amount of a polynucleotide comprising a nucleotide sequence encoding a light-responsive opsin protein to the individual, wherein the opsin protein is expressed on the plasma membrane of a population of neurons responsible for the innervation of the genitalia of the individual, wherein the opsin protein induces depolarization of the neurons expressing the opsin protein in response to light, whereby the depolarization of the neurons responsible for the innervation of the genitalia restores sexual function.

[0025] In another aspect, provided herein is a kit for treating a bladder dysfunction in an individual in need thereof, comprising: a) a polynucleotide encoding a light-responsive opsin protein, wherein the polynucleotide comprises a nucleotide sequence encoding the opsin protein comprising a sequence at least 95% identical to the sequence shown in SEQ ID NO:1, an ER export signal, and a membrane trafficking signal; and b) a light source capable of generating light in response to an external signal, wherein the light is capable of activating the opsin protein.

[0026] In another aspect, provided herein is a kit for treating a bladder dysfunction in an individual in need thereof, comprising: a) a polynucleotide encoding a light-responsive opsin protein, wherein the polynucleotide comprises a nucleotide sequence encoding the opsin protein comprising a sequence at least 95% identical to the sequence shown in SEQ ID NO:4, an ER export signal, and a membrane trafficking signal; and b) a light source capable of generating light in response to an external signal, wherein the light is capable of activating the opsin protein.

[0027] In another aspect, provided herein is a kit for treating a bladder dysfunction in an individual in need thereof, comprising: a) a polynucleotide encoding a light-responsive opsin protein, wherein the polynucleotide comprises a nucleotide sequence encoding the opsin protein comprising a sequence at least 95% identical to the sequence shown in SEQ ID NO:23, an ER export signal, and a membrane trafficking signal; and b) a light source capable of generating light in response to an external signal, wherein the light is capable of activating the opsin protein.

[0028] In still other aspects, provided herein is a kit for treating a bladder dysfunction in an individual in need thereof, comprising: a) a polynucleotide encoding a light-responsive opsin protein, wherein the polynucleotide comprises a nucleotide sequence encoding an amino acid sequence comprising a sequence at least 95% identical to one or more sequences selected from the group consisting of SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, and SEQ ID NO:11; and b) a light source capable of generating light in response to an external signal, wherein the light is capable of activating the opsin protein.

[0029] In another aspect, provided herein is a kit for treating fecal incontinence in an individual in need thereof, comprising: a) a polynucleotide encoding a light-responsive opsin protein, wherein the polynucleotide comprises a nucleotide sequence encoding the opsin protein comprising a sequence at least 95% identical to the sequence shown in SEQ ID NO:1, an ER export signal, and a membrane trafficking signal; and b) a light source capable of generating light in response to an external signal, wherein the light is capable of activating the opsin protein.

[0030] In yet another aspect, provided herein is a kit for treating fecal incontinence in an individual in need thereof, comprising: a) a polynucleotide encoding a light-responsive opsin protein, wherein the polynucleotide comprises a nucleotide sequence encoding an amino acid sequence comprising a sequence at least 95% identical to one or more sequences selected from the group consisting of SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID

NO:10, and SEQ ID NO:11; and b) a light source capable of generating light in response to an external signal, wherein the light is capable of activating the opsin protein.

[0031] In other aspects, provided herein is a kit for treating sexual dysfunction in an individual in need thereof, comprising: a) a polynucleotide encoding a light-responsive opsin protein, wherein the polynucleotide comprises a nucleotide sequence encoding an amino acid sequence comprising a sequence at least 95% identical to one or more sequences selected from the group consisting of SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, and SEQ ID NO:11; and b) a light source capable of generating light in response to an external signal, wherein the light is capable of activating the opsin protein.

BRIEF DESCRIPTION OF THE DRAWINGS

[0032] Figure 1 Figure 1 depicts schematically the various sections of the spinal cord, including the dorsolateral nucleus (DLN).

[0033] Figure 2 depicts numbers of external urinary sphincter (EUS) pudendal motor neurons expressing AAV construct-encoded opsins.

[0034] Figure 3 depicts optical stimulation of pudendal nerve expressing ChR2.

[0035] Figure 4 depicts an exemplary embodiment of optogenetic control of the EUS.

DETAILED DESCRIPTION

[0036] This invention provides, *inter alia*, methods for treating bladder dysfunction, fecal incontinence, and/or sexual dysfunction in an individual by selectively altering the electrical membrane potential of the peripheral neuronal cells that govern the filling and voiding of the urinary bladder, the contraction of the external anal sphincter, and/or the innervation of the muscles and organs of the genitalia. The inventors have developed methods to treat diseases and conditions that disrupt normal urinary, rectal, and sexual function using one or more light-responsive opsin proteins which are stably expressed in the peripheral neural cells that innervate the muscles and organs responsible for these physiological processes.

[0037] Selective control of the membrane polarization state of nerves which innervate the detrusor and external urinary sphincter (also referred to as “external urethral sphincter”) permits the bladder to fill while simultaneously allowing the external urinary sphincter to remain in a “closed” state. This can be accomplished by the selective hyperpolarization or depolarization-induced synaptic depletion of detrusor innervations via the use of light-responsive ion pumps or cation channels, respectively, and permitting the external urinary sphincter innervations to depolarize naturally into a closed state. Alternatively, if neural control of the external urinary sphincter is disrupted, damaged, or otherwise unable to generate sufficient muscular strength to close the sphincter, optogenetic or traditional electrostimulatory methods can be used in combination with optogenetic control of the detrusor to close the external urinary sphincter.

[0038] When the individual feels the urge to void or wishes to void according to a predetermined schedule, the nerves which innervate the external urinary sphincter can be selectively hyperpolarized

or subjected to depolarization-induced synaptic depletion, causing the sphincter to relax, while simultaneously ceasing the selective hyperpolarization or depolarization-induced synaptic depletion of the detrusor, permitting it to naturally depolarize and contract, thereby forcing urine out of the bladder. If the innervations of the detrusor muscle are disrupted, damaged, or otherwise unable to generate sufficient muscular strength to void the urinary bladder, however, optogenetic or electrostimulatory methods may then be used to drive depolarization of these neurons, thereby forcing the detrusor to contract and void the content of the bladder.

[0039] Thus, the methods disclosed herein can relieve or prevent many of the symptoms associated with bladder dysfunctions (for example, detrusor hyperreflexia and detrusor external sphincter dyssynergia) including, but not limited to, daytime and night time wetting, urinary retention, urinary tract and bladder infections, vesicoureteral reflux, hydroureteronephrosis, kidney stones, renal insufficiency, and/or renal failure.

[0040] Selective control of the membrane polarization state of nerves which innervate the external anal sphincter permits control of defecation and storage of stool in the rectum. This can be accomplished by the selective hyperpolarization or depolarization-induced synaptic depletion of external anal sphincter innervations via the use of light-responsive ion pumps or cation channels, respectively. When the individual feels the urge to defecate or wishes to defecate according to a predetermined schedule, the nerves which innervate the external anal sphincter can be selectively hyperpolarized or subjected to depolarization-induced synaptic depletion, causing the naturally contracted sphincter muscle to relax. When defecation is completed, optogenetic hyperpolarization or depolarization-induced synaptic depletion of external anal sphincter innervations can cease, resulting in the external anal sphincter returning to its natural, closed state. Alternatively, if neural control of the external anal sphincter is disrupted, damaged, or otherwise unable to generate sufficient muscular strength to naturally close the sphincter, optogenetic or traditional electrostimulatory methods can be used to close the external anal sphincter. Additionally, control of fecal continence can be further enhanced by simultaneous selective depolarization of the innervations of the rectum, compelling the rectum to contract and force its contents into and through the optogenetically-relaxed external anal sphincter. Thus, the methods disclosed herein can relieve or prevent fecal incontinence.

[0041] Selective control of the membrane polarization state of nerves which innervate the external genitalia of both men and women permits restoration of sexual function. This can be accomplished by the selective depolarization of one or more sacral spinal nerves and/or components of the pudendal nerve trunk to control sexual function such as—in males—erection, ejaculation, or orgasm, and—in females—vaginal lubrication or orgasm, as well as to restore tactile sensations needed to maintain sexual arousal via the use of light-responsive cation channels. Thus, the methods disclosed herein can restore sexual function.

General Techniques

[0042] The practice of the present invention will employ, unless otherwise indicated, conventional techniques of molecular biology, microbiology, cell biology, biochemistry, nucleic acid chemistry, immunology, physiology, urology, and the pathophysiology of urination 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.), Corcos & Schickik, *Textbook of the Neurogenic Bladder: Adults and Children*. (Informa Health Care, 2004), Walsh et al., *Campbell’s Urology*, 8th ed., (Saunders, 2002), *Fecal Incontinence: Diagnosis and Treatment*, (Ratto & Doglietto, eds., Springer, 2007), and Lechtenberg et al., *Sexual Dysfunction: Neurologic, Urologic, and Gynecologic Aspects* (Lippincott Williams & Wilkins, 1994).

Definitions

[0043] As used herein, “depolarization-induced synaptic depletion” occurs when continuous depolarization of a neural cell plasma membrane prevents the neural cell from sustaining high frequency action on efferent targets due to depletion of terminal vesicular stores of neurotransmitters.

[0044] An “individual” can be 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 one aspect, an individual is a human. In another aspect, an individual is a non-human animal.

[0045] Amino acid substitutions in a native protein sequence may be “conservative” or “non-conservative” and such substituted amino acid residues may or may not be one encoded by the genetic code. A “conservative amino acid substitution” is one in which the amino acid residue is replaced with an amino acid residue having a chemically similar side chain (*i.e.*, replacing an amino acid possessing a basic side chain with another amino acid with a basic side chain). A “non-conservative amino acid substitution” is one in which the amino acid residue is replaced with an amino acid residue having a chemically different side chain (*i.e.*, replacing an amino acid having a basic side chain with an amino acid having an aromatic side chain). The standard twenty amino acid “alphabet” is divided into chemical families based on chemical properties of their side chains. These

families include amino acids with basic side chains (*e.g.*, lysine, arginine, histidine), acidic side chains (*e.g.*, aspartic acid, glutamic acid), uncharged polar side chains (*e.g.*, glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine), nonpolar side chains (*e.g.*, alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan), beta-branched side chains (*e.g.*, threonine, valine, isoleucine) and side chains having aromatic groups (*e.g.*, tyrosine, phenylalanine, tryptophan, histidine).

[0046] As used herein, an “effective dosage” or “effective amount” of drug, compound, or pharmaceutical composition is an amount sufficient to effect beneficial or desired results. For prophylactic use, beneficial or desired results include results such as eliminating or reducing the risk, lessening the severity, or delaying the onset of the disease, including biochemical, histological and/or behavioral symptoms of the disease, its complications and intermediate pathological phenotypes presenting during development of the disease. For therapeutic use, beneficial or desired results include clinical results such as decreasing one or more symptoms resulting from the disease, increasing the quality of life of those suffering from the disease, decreasing the dose of other medications required to treat the disease, enhancing effect of another medication such as via targeting, delaying the progression of the disease, and/or prolonging survival. An effective dosage can be administered in one or more administrations. For purposes of this invention, an effective dosage of drug, compound, or pharmaceutical composition is an amount sufficient to accomplish prophylactic or therapeutic treatment either directly or indirectly. As is understood in the clinical context, an effective dosage of a drug, compound, or pharmaceutical composition may or may not be achieved in conjunction with another drug, compound, or pharmaceutical composition. Thus, an “effective dosage” may be considered in the context of administering one or more therapeutic agents, and a single agent may be considered to be given in an effective amount if, in conjunction with one or more other agents, a desirable result may be or is achieved.

[0047] As used herein, “treatment” or “treating” is an approach for obtaining beneficial or desired results including clinical results. For purposes of this invention, beneficial or desired clinical results include, but are not limited to, one or more of the following: decreasing symptoms resulting from the disease, increasing the quality of life of those suffering from the disease, decreasing the dose of other medications required to treat the disease, delaying the progression of the disease, and/or prolonging survival of individuals.

[0048] As used herein “sacral spinal nerve” refers to any of the mixed spinal nerves which carry motor, sensory, and autonomic signals between the spinal cord and the body originating near one of the five fused sacral bones of the vertebral column (such as any of S1, S2, S3, S4, or S5).

[0049] As used herein, the singular forms “a”, “an”, and “the” include plural references unless indicated otherwise.

[0050] 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.

Urinary Bladder Physiology

[0051] The lower urinary tract of healthy individuals has two discrete phases of activity: the storage phase, wherein urine is stored in the bladder; and the voiding phase, occurring when urine is released through the urethra. This reflex system is controlled by both a conscious signal from the brain and the firing rate of sensory fibers contained within the bladder and urethra (Yoshimura & Chancellor, 2003, *Rev. Urol.* 5 (Suppl 8): S3–S10). The bladder of an average person can hold 350 ml to 550 ml of urine. When the volume of fluid in the bladder is low, sensory receptor nerve firing is low, resulting in excitation and depolarization of the neural cells innervating the external urinary sphincter and relaxation of the detrusor muscle surrounding the urinary bladder (Blok & Holstege, *Neurosci. Lett.*, 1994, 166 (1): 93–6). When the bladder is almost full, stretch receptors lining the bladder wall trigger an increase in afferent firing, resulting in the conscious sensation of urinary urge. Generally, a person feels the need to urinate when there is approximately 200 ml of urine in the bladder. When the individual is ready to urinate, he or she consciously initiates voiding, resulting in bladder contraction and the relaxation of the external urinary sphincter. Voiding continues until the bladder empties completely, at which point the bladder relaxes and the external urinary sphincter contracts to re-initiate storage. The muscles controlling the micturition reflex are controlled by both the autonomic and somatic nervous systems. During the storage phase, the internal urethral sphincter remains tense and the detrusor muscle relaxed by sympathetic stimulation. During micturition, parasympathetic stimulation causes the detrusor muscle to contract and the internal urethral sphincter to relax. The external urinary sphincter (a.k.a. sphincter urethrae) is under somatic control and is consciously relaxed during micturition.

[0052] Neurogenic dysfunction of the urinary bladder is most often caused by diseases of or injuries to the cells of the central nervous system or the peripheral nerves involved in the control of urination. Detrusor hyperreflexia (DH) and detrusor-external sphincter dyssynergia (DSD) are two conditions that result from disruption of the central nervous system's regulation of the micturition reflex. The methods described herein may be used to alleviate the symptoms of bladder dysfunction in individuals.

Detrusor hyperreflexia

[0053] The detrusor urinate muscle, (a.k.a. detrusor muscle, muscularis propria of the urinary bladder, and muscularis propria) surrounds the urinary bladder and contracts when urinating to squeeze out urine. Otherwise, it remains relaxed to allow the bladder to fill. Detrusor hyperreflexia refers to a neurogenic condition whereby the detrusor muscle abnormally contracts which prevents

the bladder from fully emptying. Symptoms of DH range from detrusor underactivity to overactivity, depending on the site and nature of neurologic insult. For example, “detrusor hyperreflexia with impaired contractility” (DHIC) refers to overactive bladder symptoms, but the detrusor muscle is unable to produce enough pressure to permit total emptying. Therefore, the detrusor is too weak to mount an adequate contraction for proper voiding to occur. The condition is similar to urinary retention, but irritating voiding symptoms are prevalent. Additionally, “detrusor areflexia” refers to the complete inability of the detrusor to empty due to a lower motor neuron lesion (*e.g.*, to the sacral cord or peripheral nerves).

[0054] Causes of detrusor hyperreflexia are varied. Lesions to the brain in the region above the pons destroy the micturition master control center, resulting in a complete loss of voiding control. However, the primitive voiding reflexes of the lower urinary tract are still intact. Individuals sustaining such an injury exhibit urge incontinence (a.k.a. spastic bladder). In this case, the bladder empties quickly and often, with relatively low volumes of urine. Additionally, storage of urine in the bladder is difficult and individuals with this problem typically rush to the bathroom and even leak urine before reaching their destination. Often, the condition results in the disruption of sleep several times during the night, requiring the individual to make several trips to relieve the urge to void. Typical examples of brain lesions which can result in the symptoms of detrusor hyperreflexia, include, without limitation, stroke, brain tumor, Parkinson disease, Hydrocephalus, cerebral palsy, and traumatic brain injury.

[0055] Diseases or injuries of the spinal cord between the pons and the sacral spinal cord can also result in detrusor hyperreflexia. Individuals sustaining spinal injuries resulting in paraplegia or quadriplegia typically experience lower extremity spasticity. In the period initially following spinal cord trauma, the individual experiences a loss of sensation accompanied by motor paralysis with initial loss but gradual recovery of reflexes after about 6-12 weeks. This gradual recovery of the nervous system often leads to hyperstimulation of affected organs, including the detrusor muscle of the bladder. Similar to individuals with brain lesions, individuals suffering spinal cord trauma often experience urge incontinence. In addition to accidents associated with sports injuries or motor vehicles, multiple sclerosis (MS) can also cause of spinal cord disease. Additionally, children born with myelomeningocele can also exhibit neurogenic bladders leading to symptoms associated with detrusor hyperreflexia. In some aspects of the methods for treating detrusor hyperreflexia provided herein, an injury to the nervous system which causes detrusor hyperreflexia in an individual does not occur in nerve cells located inferior to the second, third, and/or fourth sacral spinal nerves (S2, S3, S4).

[0056] Diseases resulting in peripheral neuropathy can also cause symptoms associated with detrusor hyperreflexia. Diabetes mellitus and AIDS can damage or destroy the nerves which innervate the detrusor muscle of the bladder and can lead to silent and painless distention of the bladder. Patients with chronic diabetes can lose the sensation of bladder filling first, before the

bladder decompensates. Affected individuals have difficulty urinating and may also experience DHIC. Other non-limiting examples of diseases of the peripheral nervous system that can cause detrusor hyperreflexia are poliomyelitis, Guillain-Barré syndrome, severe herpes in the genitoanal area, pernicious anemia, and neurosyphilis.

[0057] Detrusor hyperreflexia is diagnosed via cystometry, which is used to evaluate bladder function. Pressure-flow studies involve the measurement of detrusor pressures while the patient is voiding to distinguish DH from detrusor acontractility. Additional diagnostic information can be obtained from: a voiding diary, urinalysis and culture, plasma electrolyte and creatinine levels or a renal tract ultrasound which includes measurement of post-void residual (PVR) urine volume.

Detrusor-external sphincter dyssynergia (DSD)

[0058] This voiding disorder is similar to detrusor hyperreflexia except that the external urinary sphincter may have paradoxical contractions simultaneously with those of the detrusor muscle when attempting to void. Normally, the detrusor muscle and the external urinary sphincter operate in synergy, such that when one is contracting (*e.g.* the sphincter during storage phase or the detrusor during voiding phase) the other is relaxed (*e.g.* the sphincter during voiding phase or the detrusor during storage phase). If both the bladder and external sphincter contract at the same time, the affected individual will sense an intense desire to urinate but may only be able to actually void a small amount. The medical term for this is detrusor-external sphincter dyssynergia because the bladder and the external sphincter are not operating in synergy. Even though the bladder is trying to force out urine, the external sphincter is tightening to prevent urine from leaving.

[0059] Both the detrusor muscle and the external urinary sphincter are innervated via nerves that originate in the sacral spinal cord. The detrusor muscle is innervated by the parasympathetic detrusor innervations arising from the sacral spinal nerves. The external urinary sphincter, on the other hand, is innervated by the external urinary sphincter innervations of the pudendal nerve. The pudendal nerve originates in the sacral plexus and derives its fibers from the ventral branches of the second, third, and fourth sacral spinal nerves (S2, S3, S4). It passes between the piriformis and coccygeus muscles and leaves the pelvis through the lower part of the greater sciatic foramen. It then crosses the spine of the ischium, and reenters the pelvis through the lesser sciatic foramen. It accompanies the internal pudendal vessels upward and forward along the lateral wall of the ischiorectal fossa, and is contained in a sheath of the obturator fascia known as the pudendal canal. The pudendal nerve gives off the inferior rectal nerves and then divides into two terminal branches: the perineal nerve, and the dorsal nerve of the penis (males) or the dorsal nerve of the clitoris (in females). The inferior anal nerves branch off shortly after passing through the greater sciatic foramen. In some aspects of the methods for treating detrusor-external sphincter dyssynergia provided herein, an injury to the nervous system which causes detrusor-external sphincter dyssynergia in an individual does not occur in nerve cells located inferior to the second, third, and/or fourth sacral spinal nerves (S2, S3, S4).

[0060] DSD is diagnosed initially from an electromyography (EMG) trace of the urethral sphincter which measures the electrical potentials generated by depolarization of muscle cells.

Videourodynamic studies are used to confirm DSD and identify other physiologic or anatomic irregularities.

Fecal Incontinence

[0061] Fecal incontinence (FI) is the loss of regular control of the bowels. Involuntary excretion and leaking are common occurrences for those affected. Fecal incontinence can be caused by damage to the nerves that control the anal sphincters or to the nerves that detect stool in the rectum. Damage to the nerves controlling the sphincter muscles may render the muscles unable to work effectively. If the sensory nerves are damaged, detection of stool in the rectum is disabled, and one will not feel the need to defecate until too late. Nerve damage can be caused by, without limitation, childbirth, long-term constipation, stroke, and diseases that cause nerve degeneration, such as diabetes and multiple sclerosis. Damage to the spinal cord may also result in fecal incontinence. Additionally, while the rectum normally stretches to hold stool until it is voluntarily released, rectal surgery, radiation treatment, and inflammatory bowel disease can cause scarring, which may result in the walls of the rectum becoming stiff and less elastic. The rectal walls are unable to stretch as much and are unable to accommodate as much stool leading to FI. Inflammatory bowel disease also can make rectal walls very irritated and thereby unable to contain stool.

[0062] The anal sphincters keep the anus closed as stool collects in the rectum. Eventually pressure on the rectal wall causes the internal anal sphincter to relax while conscious motor control over the external anal sphincter (EAS) allows stool to pass out of the body through the anus. The EAS is a flat plane of muscular fibers, elliptical in shape and intimately adherent to the integument surrounding the margin of the anus. The EAS is, like other muscles, always in a state of tonic contraction, and having no antagonistic muscle it keeps the anal canal and orifice closed. Additionally, it can be put into a condition of greater contraction under the influence of the will, so as more firmly to occlude the anal aperture. It is innervated by a branch from the fourth sacral spinal nerve (S4) and by the rectal (a.k.a inferior hemorrhoidal) branch of the pudendal nerve.

Sexual Dysfunction

[0063] Sexual dysfunction refers to a difficulty experienced by an individual or a couple during any stage of a normal sexual activity, including desire, arousal, or orgasm. There are many factors which may result in a person experiencing a sexual dysfunction which may result from emotional or physical causes. For example, sexual activity may be impacted by physical factors such as use of drugs, alcohol, nicotine, narcotics, stimulants, antihypertensives, antihistamines, and some psychotherapeutic drugs. Injuries to the back may also impact sexual activity, as would problems with an enlarged prostate gland, problems with blood supply, and nerve damage (as in spinal cord injuries). Disease, such as diabetic neuropathy, multiple sclerosis, tumors, and, rarely, tertiary syphilis, may also impact sexual activity, as would failure of various organ systems (such as the heart

and lungs), endocrine disorders (for example, hypothalamus, thyroid, pituitary, or adrenal gland problems), hormonal deficiencies (for example, low testosterone, estrogen, or androgens), and some birth defects.

[0064] For males, the skin of the penis is innervated by the dorsal nerve of the penis which is the deepest division of the pudendal nerve; it accompanies the internal pudendal artery along the ramus of the ischium, running forward along the margin of the inferior ramus of the pubis, between the superior and inferior layers of the fascia of the urogenital diaphragm. In females, the clitoris is innervated by a similar branch of the pudendal nerve known as the dorsal nerve of the clitoris. Additionally, the pudendal nerve plays a role in the innervation of the bulbospongiosus and ischiocavernosus muscles and areas around the scrotum (in males), perineum, and anus. During sexual intercourse, at sexual climax, spasms in the bulbospongiosus and ischiocavernosus result in ejaculation in the male and most of the external sensations associated with the experience of orgasm in both sexes.

Light-responsive opsin proteins

[0065] Provided herein are optogenetic-based methods for selectively hyperpolarizing or depolarizing the neurons responsible for the innervation of the detrusor muscle and the external urinary sphincter with light-responsive opsin proteins to effectively restore detrusor-sphincter synergy in individuals afflicted with bladder dysfunction (such as DH and DSD). Also provided herein are optogenetic-based methods for selectively hyperpolarizing or depolarizing the neurons responsible for the innervation of the external anal sphincter to effectively restore bowel control in individuals afflicted with fecal incontinence. Additionally, provided herein are optogenetic-based methods for selectively depolarizing the neurons responsible for the innervation of the muscles and organs of the genitalia with light-responsive opsin proteins to effectively restore sexual function in individuals afflicted with sexual dysfunction. 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. Any microbial opsin that can be used to promote neural cell membrane hyperpolarization or depolarization in response to light may be used. For example, the Halorhodopsin family of light-responsive chloride pumps (*e.g.*, NpHR, NpHR2.0, NpHR3.0, NpHR3.1) and the GtR3 proton pump can be used to promote neural cell membrane hyperpolarization in response to light. As another example, eARCH (a proton pump) can be used to promote neural cell membrane hyperpolarization in response to light. Additionally, members of the Channelrhodopsin family of light-responsive cation channel proteins (*e.g.*, ChR2, SFOs, SSFOs,

C1V1s) can be used to promote neural cell membrane depolarization or depolarization-induced synaptic depletion in response to a light stimulus.

Enhanced intracellular transport amino acid motifs

[0066] 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 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 protein. Optionally, the light-responsive protein and the one or more amino acid sequence motifs may be separated by a linker. In some embodiments, the light-responsive 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 (SEQ ID NO:12).

[0067] Trafficking sequences that are suitable for use can comprise an amino acid sequence having 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%, amino acid sequence identity to an amino acid sequence such a trafficking sequence of human inward rectifier potassium channel Kir2.1 (e.g., KSRITSEGEYIPLDQIDINV (SEQ ID NO:12)).

[0068] A trafficking sequence can have a length of from about 10 amino acids to about 50 amino acids, e.g., from about 10 amino acids to about 20 amino acids, from about 20 amino acids to about 30 amino acids, from about 30 amino acids to about 40 amino acids, or from about 40 amino acids to about 50 amino acids.

[0069] Signal sequences that are suitable for use can comprise an amino acid sequence having 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%, amino acid sequence identity to an amino acid sequence such as one of the following:

[0070] 1) the signal peptide of hChR2 (e.g., MDYGGALSAVGRELLFVTNPVVVNGS (SEQ ID NO:13))

[0071] 2) the β 2 subunit signal peptide of the neuronal nicotinic acetylcholine receptor (e.g., MAGHSNSMALFSFSLWLCSGVLGTEF (SEQ ID NO:14));

[0072] 3) a nicotinic acetylcholine receptor signal sequence (e.g., MGLRALMLWLLAAAGLVRESLQG (SEQ ID NO:15)); and

[0073] 4) a nicotinic acetylcholine receptor signal sequence (e.g., MRGTPLLLVVSLSLLQD (SEQ ID NO:16)).

[0074] A signal sequence can have a length of from about 10 amino acids to about 50 amino acids, e.g., from about 10 amino acids to about 20 amino acids, from about 20 amino acids to about 30 amino acids, from about 30 amino acids to about 40 amino acids, or from about 40 amino acids to about 50 amino acids.

[0075] Endoplasmic reticulum (ER) export sequences that are suitable for use in a modified opsin of the present disclosure include, e.g., VXXSL (where X is any amino acid) (e.g., VKESL (SEQ ID NO:17); VLGSL (SEQ ID NO:18); etc.); NANSFCYENEVALTSK (SEQ ID NO:19); FXYENE (SEQ ID NO:20) (where X is any amino acid), e.g., FCYENEV (SEQ ID NO:21); and the like. An ER export sequence can have a length of from about 5 amino acids to about 25 amino acids, e.g., from about 5 amino acids to about 10 amino acids, from about 10 amino acids to about 15 amino acids, from about 15 amino acids to about 20 amino acids, or from about 20 amino acids to about 25 amino acids.

[0076] Additional protein motifs which can enhance light-responsive protein transport to the plasma membrane of a cell are described in U.S. Patent Application No. 12/041,628, 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

[0077] 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 the neural cells which comprise the innervations of the detrusor muscle and the external urinary sphincter. In some embodiments, said one or more light-responsive chloride pumps are expressed on the plasma membrane of the nerves comprising one or more sacral spinal nerves. In other embodiments, said one or more light-responsive chloride pumps are expressed on the plasma membrane of nerves comprising the detrusor innervations arising from the sacral spinal nerves. In another embodiment, said one or more light-responsive chloride pumps are expressed on the plasma membrane of the pudendal nerve. In other embodiments, said one or more light-responsive chloride pumps are expressed on the plasma membrane of nerves comprising the external urinary sphincter innervations of the pudendal nerve.

[0078] In some aspects, said one or more light-responsive chloride pump proteins expressed on the plasma membranes of the nerve cells described above 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 nerve cell 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 630 nm. In some embodiments, the light can be at a wavelength of about 589 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.

[0079] 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 (SEQ ID NO:20), 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 (SEQ ID NO:21).

[0080] Endoplasmic reticulum (ER) export sequences that are suitable for use in a modified opsin of the present disclosure include, *e.g.*, VXXSL (where X is any amino acid) (*e.g.*, VKESL (SEQ ID NO:17); VLGSL (SEQ ID NO:18); *etc.*); NANSFCYENEVALTSK (SEQ ID NO:19); FXYENE (where X is any amino acid) (SEQ ID NO:20), *e.g.*, FCYENEV (SEQ ID NO:21); and the like. An ER export sequence can have a length of from about 5 amino acids to about 25 amino acids, *e.g.*, from about 5 amino acids to about 10 amino acids, from about 10 amino acids to about 15 amino

acids, from about 15 amino acids to about 20 amino acids, or from about 20 amino acids to about 25 amino acids.

[0081] 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 (SEQ ID NO:12).

[0082] 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 (SEQ ID NO:22). In another embodiment, the light-responsive chloride pump protein comprises an amino acid sequence at least 95% identical to SEQ ID NO:2.

[0083] 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.

[0084] 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 (SEQ ID NO:20), 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 (SEQ ID NO:21). 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 (SEQ ID NO:12). 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.

[0085] 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 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.

[0086] 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

[0087] In some aspects of the methods provided herein, one or more light-responsive proton pumps are expressed on the plasma membranes of the neural cells which comprise the innervations of the detrusor muscle and the external urinary sphincter. In some embodiments, one or more light-responsive proton pumps are expressed on the plasma membrane of the nerves comprising one or more sacral spinal nerves. In other embodiments, the one or more light-responsive proton pumps are expressed on the plasma membrane of nerves comprising the detrusor innervations arising from the sacral spinal nerves. In another embodiment, one or more light-responsive proton pumps are expressed on the plasma membrane of the pudendal nerve. In other embodiments, the one or more light-responsive proton pumps are expressed on the plasma membrane of nerves comprising the external urinary sphincter innervations of the pudendal nerve.

[0088] 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.

[0089] 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.

[0090] 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 protein in neural cells (*e.g.* the neural cells which comprise the detrusor innervations arising from the sacral spinal nerves and the external urinary sphincter innervations of the pudendal nerve).

[0091] 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.

[0092] In some embodiments, the light-responsive proton pump protein can be responsive to green or yellow light and can be derived from *Halorubrum sodomense*, wherein the proton pump protein

can be capable of mediating a hyperpolarizing current in the cell when the cell is illuminated with green or yellow light. The light can have a wavelength between about 560 and about 570 nm or can have a wavelength of about 566 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:23. 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.

[0093] 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:23 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.

[0094] 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:23. 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:23. The polynucleotides may be used for expression of the light-responsive protein in neural cells (*e.g.* the neural cells which comprise the detrusor innervations arising from the sacral spinal nerves and the external urinary sphincter innervations of the pudendal nerve).

Light-responsive cation channel proteins

[0095] In some aspects of the methods provided herein, one or more light-responsive cation channels can be expressed on the plasma membranes of the neural cells which comprise the innervations of the detrusor muscle and the external urinary sphincter. In some embodiments, one or more light-responsive cation channels can be expressed on the plasma membrane of one or more sacral spinal nerves (such as any of S1, S2, S3, S4, and/or S5). In other embodiments, the one or more light-responsive cation channels can be expressed on the plasma membrane of nerves comprising the detrusor innervations arising from the sacral spinal nerves. In another embodiment, one or more light-responsive cation channels can be expressed on the plasma membrane of the pudendal nerve. In other embodiments, the one or more light-responsive cation channels can be expressed on the plasma membrane of nerves comprising the external urinary sphincter innervations of the pudendal nerve.

[0096] In some aspects, the light-responsive 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-responsive 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-responsive 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-responsive 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-responsive cation channel. The light-responsive 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-responsive cation channel protein to regulate the polarization state of the plasma membrane of the cell. Additionally, the light-responsive cation channel 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 depolarize the plasma membrane of a neuronal cell in response to light.

[0097] In some embodiments, the light-responsive cation channel comprises a T159C substitution of the amino acid sequence set forth in SEQ ID NO:5. In some embodiments, the light-responsive cation channel comprises a L132C substitution of the amino acid sequence set forth in SEQ ID NO:5. In some embodiments, the light-responsive cation channel comprises an E123T substitution of the amino acid sequence set forth in SEQ ID NO:5. In some embodiments, the light-responsive cation channel comprises an E123A substitution of the amino acid sequence set forth in SEQ ID NO:5. In some embodiments, the light-responsive cation channel comprises a T159C substitution and an E123T substitution of the amino acid sequence set forth in SEQ ID NO:5. In some embodiments, the light-responsive cation channel comprises a T159C substitution and an E123A substitution of the amino acid sequence set forth in SEQ ID NO:5. In some embodiments, the light-responsive cation channel comprises a T159C substitution, an L132C substitution, and an E123T substitution of the amino acid sequence set forth in SEQ ID NO:5. In some embodiments, the light-responsive cation channel comprises a T159C substitution, an L132C substitution, and an E123A substitution of the amino acid sequence set forth in SEQ ID NO:5. In some embodiments, the light-responsive cation channel comprises an L132C substitution and an E123T substitution of the amino acid sequence set forth in SEQ ID NO:5. In some embodiments, the light-responsive cation channel comprises an L132C substitution and an E123A substitution of the amino acid sequence set forth in SEQ ID NO:5. In some embodiments, the light-responsive 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 hereby incorporated by reference in their entireties.

Step function opsins and stabilized step function opsins

[0099] In other embodiments, the light-responsive 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.

[00100] 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 another embodiment, the SSFO protein can comprise a C128T mutation in SEQ ID NO:5. In some embodiments, the SSFO protein comprises C128T and D156A mutations in SEQ ID NO:5.

[00101] 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.

[00102] 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 hereby incorporated by reference in their entireties.

C1V1 chimeric cation channels

[00103] In other embodiments, the light-responsive cation channel protein can be a C1V1 chimeric protein derived from the VChR1 protein of *Volvox carteri* and the ChR1 protein from *Chlamydomonas reinhardtii*, 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.

[00104] 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

[00105] In some aspects, the present disclosure provides polypeptides comprising substituted or mutated amino acid sequences, wherein the mutant polypeptide retains the characteristic light-activatable nature of the precursor C1V1 chimeric polypeptide but may also possess altered properties in some specific aspects. For example, the mutant light-responsive 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-responsive cation channels, and/or strong expression in animal cells.

[00106] Accordingly, provided herein are C1V1 chimeric light-responsive opsin 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.

[00107] 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.

[00108] 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 535 nm to about 540 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.

[00109] 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-responsive 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.

[00110] 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 hereby incorporated by reference in their entireties.

Polynucleotides

[00111] The disclosure also provides polynucleotides comprising a nucleotide sequence encoding a light-responsive 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. In some embodiments, the nucleic acid encoding a light-responsive 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 can be a promoter that is specific to motor 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-responsive 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 protein can be the EF1 α promoter, a cytomegalovirus (CMV) promoter, the CAG promoter, a synapsin-I promoter (e.g., a human synapsin-I promoter), a human synuclein 1 promoter, a human *Thy1* promoter, a calcium/calmodulin-dependent kinase II alpha (CAMKII α) promoter, or any other promoter capable of driving expression of the light-responsive opsin proteins in the peripheral neurons of mammals.

[00112] Also provided herein are vectors comprising a nucleotide sequence encoding a light-responsive 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 adeno-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.

[00113] 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.

[00114] 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 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. Methods of preparing AAV vectors in a baculovirus system are described in, *e.g.*, WO 2008/024998. AAV vectors can be self-complementary or single-stranded. 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.: 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 present disclosure 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.

[00115] In some embodiments, the vector(s) for use in the methods of the present disclosure 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 present disclosure 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

[00116] In some aspects, polynucleotides encoding the light-responsive opsin proteins disclosed herein (for example, an AAV vector) can be delivered directly to the neurons responsible for the innervation of the detrusor muscle and/or the neurons responsible for the innervation of the external urinary sphincter 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.* 73:3424-3429, 1999; Davidson et al., *PNAS*, 97:3428-3432, 2000; Davidson et al., *Nat. Genet.* 3:219-223, 1993; and Alisky & Davidson, *Hum. Gene Ther.* 11:2315-2329, 2000, 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 the neurons responsible for the innervation of the detrusor muscle by injection of the polynucleotide into the somatic motor neuron cell body of one or more sacral spinal nerves (such as any of S1, S2, S3, S4, and/or S5). In other embodiments, the polynucleotide encoding the light-responsive opsin proteins disclosed herein (for example, an AAV vector) can be delivered to the neurons responsible for the innervation of the external urinary sphincter by injection of the polynucleotide into the pudendal nerve. In another embodiment, the polynucleotide encoding the light-responsive opsin proteins disclosed herein (for example, an AAV vector) can be delivered to the neurons responsible for the innervation of the external urinary sphincter by injection of the polynucleotide into Onuf's nucleus.

[00117] In some aspects, polynucleotides encoding the light-responsive opsin proteins disclosed herein (for example, an AAV vector) can be delivered directly to the neurons responsible for the innervation of the external anal sphincter with a needle, catheter, or related device, using neurosurgical techniques known in the art, such as by stereotactic injection or fluoroscopy. In some

embodiments, the polynucleotide encoding the light-responsive opsin proteins disclosed herein (for example, an AAV vector) can be delivered to the neurons responsible for the innervation of the external anal sphincter by injection of the polynucleotide into a somatic motor neuron cell body of a sacral spinal nerve (for example, any of S1, S2, S3, S4, and/or S5). In other embodiments, the polynucleotide encoding the light-responsive opsin proteins disclosed herein (for example, an AAV vector) can be delivered to the neurons responsible for the innervation of the external anal sphincter by injection of the polynucleotide into the pudendal nerve or by injection of the polynucleotide into Onuf's nucleus. In other embodiments, the polynucleotide encoding the light-responsive opsin proteins disclosed herein (for example, an AAV vector) can be delivered to the neurons responsible for the innervation of the rectum by injection of the polynucleotide in a somatic motor neuron cell body of a sacral spinal nerve (for example, any of S1, S2, S3, S4, and/or S5) or by injection into the inferior hypogastric plexus.

[00118] In some aspects, polynucleotides encoding the light-responsive opsin proteins disclosed herein (for example, an AAV vector) can be delivered directly to the neurons responsible for the innervation of the muscles and organs of the male and female genitalia with a needle, catheter, or related device, using neurosurgical techniques known in the art, such as by stereotactic injection or fluoroscopy. In some embodiments, the polynucleotide encoding the light-responsive opsin proteins disclosed herein (for example, an AAV vector) can be delivered to the neurons responsible for the innervation of the muscles and organs of the male and female genitalia by injection of the polynucleotide into a somatic motor neuron cell body of a sacral spinal nerve (for example, any of S1, S2, S3, S4, and/or S5). In other embodiments, the polynucleotide encoding the light-responsive opsin proteins disclosed herein (for example, an AAV vector) can be delivered to the neurons responsible for the innervation of the muscles and organs of the male and female genitalia by injection of the polynucleotide into the pudendal nerve or by injection of the polynucleotide into Onuf's nucleus.

[00119] 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.

[00120] In another aspect, the polynucleotide encoding the light-responsive opsin proteins disclosed herein (for example, an AAV vector) can be delivered directly to the detrusor muscle of the bladder and/or the muscles responsible for the contraction of the external urinary sphincter. Because of the limitations inherent in injecting viral vectors directly into the specific cell bodies which innervate 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 entirety).

[00121] Accordingly, in some embodiments, the polynucleotides encoding the light-responsive opsin proteins disclosed herein (for example, an AAV vector) can be delivered to the neurons responsible for the innervation of the external urinary sphincter by injection of the polynucleotide expression vector directly into the muscles responsible for contraction of the external urinary sphincter. In another embodiment, the polynucleotides encoding the light-responsive opsin proteins disclosed herein (for example, an AAV vector) can be delivered to the neurons responsible for the innervation of the detrusor muscle by injection of the polynucleotide expression vector directly into the detrusor muscle. In other embodiments, the polynucleotides encoding the light-responsive opsin proteins disclosed herein (for example, an AAV vector) can be delivered to the neurons responsible for the innervation of the external anal sphincter by injection of the polynucleotide expression vector directly into the muscles responsible for contraction of the external anal sphincter. In other embodiments, the polynucleotides encoding the light-responsive opsin proteins disclosed herein (for example, an AAV vector) can be delivered to the neurons responsible for the innervation of the rectum by injection of the polynucleotide expression vector directly into the muscles responsible for contraction of the rectum. In yet another embodiment, the polynucleotides encoding the light-responsive opsin proteins disclosed herein (for example, an AAV vector) can be delivered to the neurons responsible for the innervation of the male and female genitalia by injection of the polynucleotide expression vector directly into the muscles and organs responsible for normal sexual function including, but not limited to, the bulbospongiosus and ischiocavernosus muscles of the penis, the skin of the penis, the perineal muscles, or the clitoris.

Light and Electrical Sources

[00122] In some aspects of the present disclosure, the light-responsive opsin proteins disclosed herein can be activated by an implantable light source (such as a light cuff) or an implantable electrode placed around or near nerve fibers expressing the light-responsive opsin proteins or nerves controlling the muscles of the bladder, external urinary sphincter, external anal sphincter, rectum, and/or the male or female genitalia. Electrode cuffs and electrodes surgically placed around or near nerves for use in electrical stimulation of those nerves are well known in the art (*See*, for example, U.S. Pat. Nos. 4,602,624, 7,142,925 and 6,600,956 as well as U.S. Patent Publication Nos. 2008/0172116 and 2010/0094372, the disclosures of each of which are hereby incorporated by reference in their entirety). The light sources (such as a light cuff) or electrodes of the present invention can be comprised of any useful composition or mixture of compositions, such as platinum or stainless steel, as are known in the art, and may be of any useful configuration for stimulating the light-responsive opsin proteins disclosed herein or nerves controlling the muscles and organs of the bladder, external urinary sphincter external anal sphincter, and/or male or female genitalia.

[00123] The electrodes or implantable light source (such as a light cuff) may be placed around or near a nerve, such as, but not limited to, the detrusor innervations arising from the sacral spinal nerves, the external urinary sphincter innervations of the pudendal nerve, the external anal sphincter innervations arising from the sacral spinal nerves and/or the inferior rectal nerve (a.k.a inferior hemorrhoidal nerve) arising from the pudendal nerve, the innervations of the rectum arising from the sacral spinal nerves and/or the inferior hypogastric plexus, the dorsal nerve of the penis arising from the pudendal nerve, and/or the dorsal nerve of the clitoris arising from the pudendal nerve. The nerve fibers responsible for the innervation of the aforementioned muscles and/or organs can be identified by clinicians prior to placing the electrode or implantable light source around or near the nerve fibers using known techniques in the art. For example, nerve fibers may be stimulated with brief pulses of electricity and the location of the muscular contractions caused by said electrical stimulation observed to ensure correct placement of the electrode or implantable light source. The electrodes or implantable light source are well suited to be used in conjunction with the pudendal nerve in particular, either on the pudendal nerve trunk or its afferent or efferent branches away from the sacral root (from whence the pudendal nerve originates), that innervate the muscles and organs of the lower urinary tract, the bowel, the perineum and the genitals. The pudendal nerve trunk and its branches are spaced away from the sacral root and spinal column. Consequently, they are surgically accessible from the front of the body and do not require complex surgical procedures on, in, or near the spinal column or entailing dorsal rhizotomy. In another embodiment, a light source may be placed near the ventral roots of the desired nerve targets. In another embodiment, a light source comprises a fiber optic cable placed near a desired nerve target that is surgically exposed.

[00124] In some embodiments, the implantable light source (such as a light cuff) does not completely surround the nerve, but, rather, can have a U-shape. In another embodiment, the implantable light source can have an attachment arm that can be used to guide the implantable light source (such as a light cuff) to the nerve fibers (*e.g.*, the detrusor innervations arising from the sacral spinal nerves and/or the external urinary sphincter innervations of the pudendal nerve). The attachment arm can be removed following implantation of the light source or can be left in place to fix the position of the light source in proximity to the nerve fibers of interest.

[00125] The implantable light source (such as a light cuff) can comprise an inner body, the inner body having at least one means for generating light which is configured to a power source. In some embodiments, the power source can be an internal battery for powering the light-generating means. In another embodiment, the implantable light source can comprise an external antenna for receiving wirelessly transmitted electromagnetic energy from an external source for powering the light-generating means. 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 light-generating means of the implantable light source (such as a light cuff). In

one embodiment, the light-generating means is controlled by an integrated circuit produced using semiconductor or other processes known in the art.

[00126] In some aspects, the light means can be a light emitting diode (LED). In some embodiments, the LED can generate blue and/or green light. In other embodiments, the LED can generate amber and/or yellow light. In some embodiments, several micro LEDs are embedded into the inner body of the implantable light source (such as a light cuff). In other embodiments, the light-generating means is a solid state laser diode or any other means capable of generating light. The light generating means can generate light having an intensity sufficient to activate the light-responsive opsin proteins expressed on the plasma membrane of the nerves in proximity to the light source (such as a light cuff). In some embodiments, the light-generating means produces light having an intensity of any of about 0.05 mW/mm², 0.1 mW/mm², 0.2 mW/mm², 0.3 mW/mm², 0.4 mW/mm², 0.5 mW/mm², about 0.6 mW/mm², about 0.7 mW/mm², about 0.8 mW/mm², about 0.9 mW/mm², about 1.0 mW/mm², about 1.1 mW/mm², about 1.2 mW/mm², about 1.3 mW/mm², about 1.4 mW/mm², about 1.5 mW/mm², about 1.6 mW/mm², about 1.7 mW/mm², about 1.8 mW/mm², about 1.9 mW/mm², about 2.0 mW/mm², about 2.1 mW/mm², about 2.2 mW/mm², about 2.3 mW/mm², about 2.4 mW/mm², about 2.5 mW/mm², about 3 mW/mm², about 3.5 mW/mm², about 4 mW/mm², about 4.5 mW/mm², about 5 mW/mm², about 5.5 mW/mm², about 6 mW/mm², about 7 mW/mm², about 8 mW/mm², about 9 mW/mm², or about 10 mW/mm², inclusive, including values in between these numbers. In other embodiments, the light-generating means produces light having an intensity of at least about 100 Hz.

[00127] In some aspects, the light-generating means 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 light-generating means on the light source through electromagnetic coupling between the transmitting coil on the external controller and the external antenna of the implantable light source (such as a light cuff). The transmitting coil can establish an electromagnetic coupling with the external antenna of the implantable light source when in proximity thereof, for supplying power to the light-generating means and for transmitting one or more control signals to the implantable light source. In some embodiments, the electromagnetic coupling between the transmitting coil of the external controller and the external antenna of the implantable light source (such as a light cuff) can be radio-frequency magnetic inductance coupling. 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, infrared, or a biomedical telemetry system (*See, e.g.,* Kiourti, “Biomedical Telemetry: Communication between Implanted Devices and the External World, *Opticon* 1826, (8): Spring, 2010).

[00128] In some aspects of the present disclosure, more than one of the detrusor innervations arising from the sacral spinal nerves, the external urinary sphincter innervations of the pudendal nerve, the external anal sphincter innervations of the pudendal or sacral spinal nerves can express light-responsive pumps that promote nerve hyperpolarization when activated with light. In some embodiments, the light-generating means of the implantable light source placed around or near the detrusor innervations arising from the sacral spinal nerves generates light continuously when the bladder is storing urine while, at the same time, the implantable light source (such as a light cuff) placed around or near the external urinary sphincter innervations of the pudendal nerve does not generate light. In another aspect, the light-generating means of the implantable light source (such as a light cuff) placed around or near the detrusor innervations arising from the sacral spinal nerves does not generate light when the bladder is voiding while, at the same time, the implantable light source (such as a light cuff) placed around or near the external urinary sphincter innervations of the pudendal nerve can generate light continuously. In some embodiments, a first control signal generated by the external controller can activate the light generating means of the implantable light source (such as a light cuff) placed around or near the detrusor innervations arising from the sacral spinal nerves while simultaneously deactivating the implantable light source (such as a light cuff) placed around or near the external urinary sphincter innervations of the pudendal nerve. In another embodiment, a second control signal generated by the external controller can deactivate the light generating means of the implantable the light source (such as a light cuff) placed around or near the detrusor innervations arising from the sacral spinal nerves while simultaneously activating the implantable light source (such as a light cuff) placed around or near the external urinary sphincter innervations of the pudendal nerve. In another embodiment, a control signal generated by the external controller can activate the light generating means of the implantable the light source (such as a light cuff) placed around or near the external anal sphincter innervations arising from the pudendal or sacral spinal nerves.

[00129] In some aspects of the present disclosure, the detrusor innervations arising from the sacral spinal nerves, the external urinary sphincter innervations of the pudendal nerve, and/or the external anal sphincter innervations of the pudendal or sacral spinal nerves can express light-responsive cation channels that promote nerve depolarization-induced synaptic depletion when activated with light. In some embodiments, the light-generating means of the implantable light source placed around or near the detrusor innervations arising from the sacral spinal nerves does not generate light when the bladder is storing urine while, at the same time, the implantable light source (such as a light cuff)

placed around or near the external urinary sphincter innervations of the pudendal nerve can generate light having an intensity of at least 100 Hz continuously. In another aspect, the light-generating means of the implantable light source (such as a light cuff) placed around or near the detrusor innervations arising from the sacral spinal nerves generates light having an intensity of at least 100 Hz when the bladder is voiding while, at the same time, the implantable light source (such as a light cuff) placed around or near the external urinary sphincter innervations of the pudendal nerve does not generate light. In another aspect, the light-generating means of the implantable light source (such as a light cuff) placed around or near the external anal sphincter innervations arising from the pudendal or sacral spinal nerves generates light having an intensity of at least 100 Hz when the external anal sphincter is relaxing. In some embodiments, a first control signal generated by the external controller can activate the light generating means of the implantable light source (such as a light cuff) placed around or near the detrusor innervations arising from the sacral spinal nerves while simultaneously deactivating the implantable light source (such as a light cuff) placed around or near the external urinary sphincter innervations of the pudendal nerve. In another embodiment, a second control signal generated by the external controller can deactivate the light generating means of implantable the light source (such as a light cuff) placed around or near the detrusor innervations arising from the sacral spinal nerves while simultaneously activating the implantable light source (such as a light cuff) placed around or near the external urinary sphincter innervations of the pudendal nerve. In another embodiment, a control signal generated by the external controller can deactivate the light generating means of implantable the light source (such as a light cuff) placed around or near the external anal sphincter innervations arising from the pudendal or sacral spinal nerves.

[00130] In some aspects of the present disclosure, the innervations of the muscles or organs of the male or female genitalia arising from the pudendal nerve or one or more sacral spinal nerves can express light-responsive cation channel proteins that promote nerve depolarization when activated with light. In some embodiments, the light-generating means of the implantable light source placed around or near the innervations of the male or female genitalia arising from the pudendal or sacral spinal nerves generates light continuously when the individual wishes to engage in sexual activity such as, but not limited to, erection, orgasm, ejaculation, and/or vaginal lubrication. In some embodiments, the light-generating means of the implantable light source placed around or near the innervations of the male or female genitalia arising from the pudendal or sacral spinal nerves generates light continuously when the individual wishes to restore tactile sensations in the genital area required to maintain sexual arousal. In some embodiments, a first control signal generated by the external controller can activate the light generating means of the implantable light source (such as a light cuff) placed around or near the innervations of the muscles or organs of the male or female genitalia arising from the pudendal or sacral spinal nerves. In another embodiment, a second control signal generated by the external controller can deactivate the light generating means of implantable

the light source (such as a light cuff) placed around or near the innervations of the muscles or organs of the male or female genitalia arising from the pudendal or sacral spinal nerves.

[00131] In other aspects of the present disclosure, a combination of nerve stimulation with light-responsive opsin proteins and electricity can be used to restore urinary and/or fecal continence. In some embodiments, the detrusor innervations arising from the sacral spinal nerves can express any of the light-responsive opsin proteins described herein and an electrode can be placed around or near the external urinary sphincter innervations of the pudendal nerve. In some embodiments, the external anal sphincter innervations arising from the sacral spinal nerves and/or the pudendal nerves can have an electrode placed around or near the external anal sphincter innervations arising from the pudendal nerve and/or the sacral spinal nerves. In other embodiments, an electrode can be placed around or near the detrusor innervations arising from the sacral spinal nerves while the external urinary sphincter innervations of the pudendal nerve can express any of the light-responsive opsin proteins described herein. In some embodiments, a first control signal generated by the external controller can activate the light generating means of the implantable light source (such as a light cuff) placed around or near the detrusor innervations arising from the sacral spinal nerves while simultaneously activating an electrode placed around or near the external urinary sphincter innervations of the pudendal nerve. In another embodiment, a second control signal generated by the external controller can deactivate the light generating means of the implantable light source (such as a light cuff) placed around or near the detrusor innervations arising from the sacral spinal nerves while simultaneously deactivating an electrode placed around or near the external urinary sphincter innervations of the pudendal nerve. In another embodiment, a first control signal generated by the external controller can deactivate an electrode placed around or near the detrusor innervations arising from the sacral spinal nerves while simultaneously activating a light generating means of the implantable light source (such as a light cuff) placed around or near the external urinary sphincter innervations of the pudendal nerve. In yet another embodiment, a second control signal generated by the external controller can activate an electrode placed around or near the detrusor innervations arising from the sacral spinal nerves while simultaneously deactivating a light generating means of the implantable light source (such as a light cuff) placed around or near the external urinary sphincter innervations of the pudendal nerve.

[00132] Examples of light stimulation devices, including light source (such as a light cuff), 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

[00133] The present invention is directed to methods for inhibiting the symptoms (disabilities, impairments) associated with bladder dysfunction, fecal incontinence (FI), and/or sexual dysfunction. Particularly, the methods provided herein are directed towards treating and/or alleviating the

symptoms associated with detrusor hyperreflexia (DH) and/or detrusor external sphincter dyssynergia (DSD). As such, it is not required that physiological damage or all effects of the condition be entirely reversed, although the effects of the presently disclosed methods likely extend to a significant therapeutic benefit for the patient. As such, a therapeutic benefit is not necessarily a complete cure for DH, DSD, FI, and/or sexual dysfunction, but rather, can encompass a result which includes reducing or preventing the symptoms or physiological damage resulting from these conditions, reducing or preventing the occurrence of such symptoms or damage (either quantitatively or qualitatively), reducing the severity of such symptoms or physiological effects, and/or enhancing the recovery of the patient after experiencing a cause of DH, DSD, FI, and/or sexual dysfunction (for example, but not limited to, spinal cord injury and multiple sclerosis).

[00134] Specifically, the methods of the present invention may prevent damage associated with prolonged DH, DSD, FI, and/or sexual dysfunction, and/or reduce or alleviate symptoms of or conditions associated with (resulting from) these conditions. As such, protecting an individual from the physiological effects or symptoms resulting from DH, DSD, FI, and/or sexual dysfunction includes both preventing or reducing the occurrence and/or severity of the effects of the symptoms and treating a patient in which the symptoms are already occurring or beginning to occur. A beneficial effect can easily be assessed by one of ordinary skill in the art and/or by a trained clinician who is treating the patient. For example, many of the methods described above for the diagnosis of DH and/or DSD can be used to evaluate the patient before and after treatment using a method of the present invention to assess the success of the treatment. In some embodiments, there is a positive or beneficial difference in the severity or occurrence of at least one clinical or biological score, value, or measure used to evaluate such patients in those who have been treated with the methods of the present invention as compared to those that have not.

Hyperpolarization or depolarization-induced synaptic depletion with light-responsive opsin proteins for treating bladder dysfunction and/or fecal incontinence

Bladder dysfunction

[00135] Accordingly, provided herein is a method for treating a bladder dysfunction in an individual in need thereof, the method comprising administering an effective amount of a polynucleotide comprising a nucleotide sequence encoding a light-responsive opsin protein to the individual, wherein the light-responsive opsin protein is expressed on the plasma membrane of a population of neurons responsible for the innervation of the detrusor muscle and/or a population of neurons responsible for the innervation of the external urinary sphincter muscle in the individual, wherein the light-responsive opsin protein is capable of inducing hyperpolarization of the neurons expressing the light-responsive opsin protein in response to light, whereby the hyperpolarization of the neurons responsible for the innervation of the detrusor muscle permits storage of urine in the bladder and the hyperpolarization of the neurons responsible for the innervation of the external urinary sphincter muscle permits voiding urine from the bladder. Any polynucleotides described herein that encode a

light-responsive opsin protein capable of inducing hyperpolarization may be administered. In some embodiments, the bladder dysfunction is DH and/or DSD. In other embodiments, the population of neurons responsible for the innervation of the detrusor muscle and/or a population of neurons responsible for the innervation of the external urinary sphincter muscle are hyperpolarized by exposure to amber light. An exemplary embodiment of a subject method is depicted schematically in Figure 4.

[00136] When illuminated by an implantable light source (such as a light cuff) placed around or near the detrusor innervations arising from the sacral spinal nerves, the light-responsive opsin proteins expressed on the plasma membranes of the detrusor innervations maintain a hyperpolarized neural membrane potential preventing contraction of the detrusor muscle and permitting the bladder to fill. When the light-responsive opsin proteins expressed on the detrusor innervations arising from the sacral spinal nerves are not illuminated by the implantable light source, the detrusor muscle is able to contract due to the recovery of normal neural membrane potential and the pressure generated facilitates voiding. Similarly, when illuminated by an implantable light source placed around or near the external urinary sphincter innervations of the pudendal nerve, the light-responsive opsin proteins expressed on the plasma membranes of the external urinary sphincter innervations maintain a hyperpolarized neural membrane potential, preventing contraction of the external urinary sphincter and permitting the individual to void. When the light-responsive opsin proteins expressed on the external urinary sphincter innervations of the pudendal nerve are not illuminated by the implantable light source, the external urinary sphincter contracts and permits the bladder to fill.

[00137] Also provided herein is a method for treating a bladder dysfunction in an individual in need thereof, the method comprising administering an effective amount of a polynucleotide comprising a nucleotide sequence encoding a light-responsive opsin protein to the individual, wherein the light-responsive opsin protein is expressed on the plasma membrane of a population of neurons responsible for the innervation of the detrusor muscle and/or a population of neurons responsible for the innervation of the external urinary sphincter muscle in the individual, wherein the light-responsive opsin protein is capable of causing depolarization-induced synaptic depletion of the neurons expressing the light-responsive protein in response to light, whereby the depolarization-induced synaptic depletion of the neurons responsible for the innervation of the detrusor muscle permits storage of urine in the bladder and the depolarization-induced synaptic depletion of the neurons responsible for the innervation of the external urinary sphincter muscle permits voiding urine from the bladder. Any polynucleotides described herein that encode a light-responsive opsin protein capable of causing depolarization-induced synaptic depletion may be administered. In some embodiments, the bladder dysfunction is DH and/or DSD. In other embodiments, the population of neurons responsible for the innervation of the detrusor muscle and/or a population of neurons responsible for the innervation of the external urinary sphincter muscle can be depolarized to the

point of synaptic depletion by exposure to blue, green, yellow, orange, or red light. In some embodiments, the light has an intensity of at least about 100 Hz.

[00138] When illuminated by an implantable light source (such as a light cuff) placed around or near the detrusor innervations arising from the sacral spinal nerves, the light-responsive opsin proteins expressed on the plasma membranes of the detrusor innervations provoke depolarization-induced synaptic depletion of the neural cells, thereby preventing contraction of the detrusor muscle and permitting the bladder to fill. When the light-responsive opsin proteins expressed on the detrusor innervations arising from the sacral spinal nerves are not illuminated by the implantable light source, the detrusor muscle is able to contract due to the recovery of transsynaptic vesicles and the pressure generated facilitates voiding. Similarly, when illuminated by an implantable light source placed around or near the external urinary sphincter innervations of the pudendal nerve, the light-responsive opsin proteins expressed on the plasma membranes of the external urinary sphincter innervations cause depolarization-induced synaptic depletion of those neurons, preventing contraction of the external urinary sphincter and permitting the individual to void. When the light-responsive opsin proteins expressed on the external urinary sphincter innervations of the pudendal nerve are not illuminated by the implantable light source, the external urinary sphincter contracts and permits the bladder to fill.

[00139] In some aspects, the individual externally controls the polarization state of the neurons responsible for the innervation of the detrusor muscle and/or neurons responsible for the innervation of the external urinary sphincter by activating the light means from one or more light sources (such as a light cuff) that surrounds or is located near one of the sacral spinal nerves (such as any of S1, S2, S3, S4, or S5) and/or the pudendal nerve. In another embodiment, the individual selectively changes the membrane polarization state of the neurons responsible for the innervation of the detrusor muscle and/or neurons responsible for the innervation of the external urinary sphincter by activating the light source surrounding or located near the pudendal nerve while simultaneously deactivating the light source surrounding or located near the sacral spinal nerves when the individual experiences the need and/or urge to void or wishes to void according to a predetermined schedule. In another embodiment, the individual is a human.

[00140] In some aspects, both the population of neurons responsible for the innervation of the detrusor muscle and the population of neurons responsible for the innervation of the external urinary sphincter muscle in the individual can be transfected with an expression vector comprising any of the polynucleotides described above. In some embodiments, the expression vector can be a viral vector such as any of the viral expression vectors described above. In some aspects, the population of neurons responsible for the innervation of the detrusor muscle can be transfected by injection of the expression vector into the somatic motor neuron cell body of a sacral spinal nerve (such as any of S1, S2, S3, S4, and/or S5). In other aspects, the population of neurons responsible for the innervation of the external urinary sphincter muscle is transfected by injection of the expression vector into Onuf's

nucleus. In some aspects, one or more light sources (such as a light cuff) capable of generating light in response to an external signal, such as those described above, are surgically placed around or near the detrusor innervations arising from the sacral spinal nerves and around the external urinary sphincter innervations of the pudendal nerve.

Fecal incontinence

[00141] Also, provided herein is a method for treating a fecal incontinence in an individual in need thereof, the method comprising administering an effective amount of a polynucleotide comprising a nucleotide sequence encoding a light-responsive opsin protein to the individual, wherein the light-responsive opsin protein is expressed on the plasma membrane of a population of neurons responsible for the innervation of the external anal sphincter muscle in the individual, wherein the light-responsive opsin protein is capable of inducing hyperpolarization of the neurons expressing the light-responsive protein in response to light, whereby the hyperpolarization of the neurons responsible for the innervation of the external anal sphincter muscle permits relaxation of the sphincter. Any polynucleotides described herein that encode a light-responsive opsin protein capable of inducing hyperpolarization may be administered. In other embodiments, the population of neurons responsible for the innervation of the external anal sphincter muscle are hyperpolarized by exposure to amber light.

[00142] When illuminated by an implantable light source (such as a light cuff) placed around or near the external anal sphincter innervations of the pudendal nerve and/or one or more sacral spinal nerves (such as any of S1, S2, S3, S4, and/or S5), the light-responsive opsin proteins expressed on the plasma membranes of the external anal sphincter innervations maintain a hyperpolarized neural membrane potential, preventing contraction of the external anal sphincter and permitting the individual to defecate. When the light-responsive opsin proteins expressed on the external anal sphincter innervations of the pudendal nerve and/or the sacral spinal nerves are not illuminated by the implantable light source, the external anal sphincter naturally contracts and permits the storage of stool in the bowel.

[00143] Also provided herein is a method for treating fecal incontinence in an individual in need thereof, the method comprising administering an effective amount of a polynucleotide comprising a nucleotide sequence encoding a light-responsive opsin protein to the individual, wherein the light-responsive opsin protein is expressed on the plasma membrane of a population of neurons responsible for the innervation of the of the external anal sphincter muscle in the individual, wherein the light-responsive opsin protein is capable of causing depolarization-induced synaptic depletion of the neurons expressing the light-responsive protein in response to light, whereby the depolarization-induced synaptic depletion of the neurons responsible for the innervation of the external anal sphincter muscle permits relaxation of the sphincter. Any polynucleotides described herein that encode a light-responsive opsin protein capable of inducing depolarization-induced synaptic depletion of the neurons may be administered. In other embodiments, the population of neurons

responsible for the innervation of the external anal sphincter muscle can be depolarized to the point of synaptic depletion by exposure to blue, green, yellow, orange, or red light. In some embodiments, the light can have an intensity of at least about 100 Hz.

[00144] When illuminated by an implantable light source placed around or near the external anal sphincter innervations arising from the pudendal and/or sacral spinal nerves, the light-responsive opsin proteins expressed on the plasma membranes of the external anal sphincter innervations cause depolarization-induced synaptic depletion of those neurons, preventing contraction of the external anal sphincter and permitting the individual to defecate. When the light-responsive opsin proteins expressed on the external anal sphincter innervations of the pudendal nerve and/or the sacral spinal nerves are not illuminated by the implantable light source, the external anal sphincter naturally contracts and permits the rectum to store stool.

[00145] In some aspects, the individual externally controls the hyperpolarization state of the neurons responsible for the innervation of the external anal sphincter by activating the light means from one or more light sources (such as a light cuff) that surrounds or is located near the sacral spinal nerves and/or the pudendal nerve. In another embodiment, the individual selectively changes the membrane polarization state of the neurons responsible for the innervation of the external anal sphincter by activating the light source surrounding or located near the pudendal nerve and/or the sacral spinal nerves when the individual experiences the need and/or urge to defecate or wishes to defecate according to a predetermined schedule. In another embodiment, the individual is a human.

In some aspects, the population of neurons responsible for the innervation of the external anal sphincter muscle in the individual can be transfected with an expression vector comprising any of the polynucleotides described above. In some embodiments, the expression vector can be a viral vector such as any of the viral expression vectors described above. In some aspects, the population of neurons responsible for the innervation of the external anal sphincter muscle is transfected by injection of the expression vector into a sacral spinal nerve (such as any of S1, S2, S3, S4, and/or S5). In other aspects, the population of neurons responsible for the innervation of the external anal sphincter muscle is transfected by injection of the expression vector into the pudendal nerve or by injection of the expression vector into Onuf's nucleus. In some aspects, one or more light sources (such as a light cuff) capable of generating light in response to an external signal, such as those described above, are surgically placed around or near the external anal sphincter innervations arising from the pudendal nerve and/or the sacral spinal nerves.

Depolarization with light-responsive opsin proteins or electrostimulation to drive muscular contraction

Bladder dysfunction

[00146] Sphincter muscles exist in a naturally contracted (tonic) state due to the fact that, unlike most skeletal muscles, there is no other muscle to oppose sphincter contraction (*e.g.*, the biceps muscle is in opposition to the triceps muscle). However, in some situations, the cells or neurons

located upstream of sites of sphincter muscle innervation may be damaged, nonfunctional, or unable to generate a muscle contraction strong enough to expel urine from the bladder, to close the external urinary sphincter, or to close the external anal sphincter. Under these circumstances, depolarization of these peripheral nerves via the use of light-responsive opsin proteins or traditional electrostimulation can be used to restore urinary function and/or fecal continence and achieve “on/off” control of the detrusor muscle, urinary sphincter, and/or anal sphincter, respectively.

[00147] Accordingly, provided herein is a method for treating a bladder dysfunction in an individual in need thereof, the method comprising administering effective amounts of polynucleotide sequences (such as any of the polynucleotide sequences disclosed above) encoding a first light-responsive opsin protein and a second light-responsive opsin protein to the individual, wherein the first light-responsive opsin protein and the second light-responsive opsin protein are expressed on the plasma membrane of a population of neurons responsible for the innervation of the detrusor muscle and/or a population of neurons responsible for the innervation of the external urinary sphincter muscle in the individual, wherein the first light-responsive opsin protein is capable of inducing hyperpolarization of the neurons expressing the first light-responsive protein in response to a first wavelength of light, whereby the hyperpolarization of the neurons responsible for the innervation of the detrusor muscle permits storage of urine in the bladder and the hyperpolarization of the neurons responsible for the innervation of the external urinary sphincter muscle permits voiding urine from the bladder, wherein the second light-responsive opsin protein is capable of inducing depolarization of the neurons expressing the second light-responsive protein in response to a second wavelength of light, whereby the depolarization of the neurons responsible for the innervation of the detrusor muscle permits voiding of urine from the bladder and the depolarization of the neurons responsible for the innervation of the external urinary sphincter muscle permits the closing of the sphincter permitting the storage of urine. In some embodiments, the bladder dysfunction is DH and/or DSD.

[00148] When illuminated by light having a first wavelength provided by an implantable light source (such as a light cuff) placed around or near the detrusor innervations arising from the sacral spinal nerves, the first light-responsive opsin proteins expressed on the plasma membranes of the detrusor innervations hyperpolarize the innervations, thereby preventing contraction of the detrusor muscle and permitting the bladder to fill. When the second light-responsive opsin proteins expressed on the detrusor innervations arising from the sacral spinal nerves are illuminated by light having a second wavelength provided by the implantable light source, the detrusor muscle is able to contract due to the depolarization of the detrusor innervations. Similarly, when illuminated by light having a first wavelength provided by an implantable light source (such as a light cuff) placed around or near the external urinary sphincter innervations of the pudendal nerve, the first light-responsive opsin proteins expressed on the plasma membranes of the external urinary sphincter innervations hyperpolarize those neurons, preventing contraction of the external urinary sphincter and permitting the individual to void. When the second light-responsive opsin proteins expressed on the external

urinary sphincter innervations of the pudendal nerve are illuminated by light having a second wavelength provided by the implantable light source, the external urinary sphincter contracts due to depolarization of the innervations, permitting the storage of urine in the bladder.

Fecal incontinence

[00149] Additionally, provided herein is a method for treating fecal incontinence in an individual in need thereof, the method comprising administering effective amounts of polynucleotide sequences (such as any of the polynucleotide sequences disclosed above) encoding a first light-responsive opsin protein and a second light-responsive opsin protein to the individual, wherein the first light-responsive opsin protein and the second light-responsive opsin protein are expressed on the plasma membrane of a population of neurons responsible for the innervation of the external anal sphincter muscle in the individual, wherein the first light-responsive opsin protein is capable of inducing hyperpolarization of the neurons expressing the first light-responsive protein in response to a first wavelength of light, whereby the hyperpolarization of the neurons responsible for the innervation of the external anal sphincter muscle permits relaxation of the sphincter permitting defecation, wherein the second light-responsive opsin is capable of inducing depolarization of the neurons expressing the second light-responsive protein in response to a second wavelength of light, whereby the depolarization of the neurons responsible for the innervation of the external anal sphincter muscle permits the closing of the sphincter permitting the storage of stool in the bowel.

[00150] When illuminated by light having a first wavelength provided by an implantable light source (such as a light cuff) placed around or near the innervations of the external anal sphincter arising from the pudendal nerve and/or the sacral spinal nerves, the first light-responsive opsin proteins expressed on the plasma membranes of the external anal sphincter innervations hyperpolarize the innervations, thereby preventing contraction of the sphincter muscle and permitting the sphincter to relax. When the second light-responsive opsin proteins expressed on the plasma membranes of the external anal sphincter innervations arising from the sacral spinal nerves and/or the pudendal nerve are illuminated by light having a second wavelength provided by the implantable light source, the sphincter muscle is able to contract due to the depolarization of the sphincter innervations, thereby permitting the storage of stool in the rectum.

[00151] In another aspect, the method for treating fecal incontinence further comprises administering an effective amount of a polynucleotide (such as any of the polynucleotide sequences disclosed above) comprising a nucleotide sequence encoding a light-responsive opsin protein to the individual, wherein the light-responsive opsin protein is expressed on the plasma membrane of a population of neurons responsible for the innervation of the muscles responsible for the contraction of the rectum in the individual, wherein the light-responsive opsin protein is capable of inducing depolarization of the neurons expressing the light-responsive protein in response to light, whereby the depolarization of the neurons responsible for the innervation of the muscles responsible for the contraction of the rectum facilitates defecation. In some aspects, the individual externally controls

the depolarization state of the neurons responsible for the innervation of the muscles responsible for the contraction of the rectum by activating the light means from one or more light sources (such as a light cuff or any of the light sources described herein) that surrounds or is located near the sacral spinal nerves and/or nerve fibers arising from the inferior hypogastric plexus. In another embodiment, the individual selectively changes the membrane polarization state of the neurons responsible for the innervation of the muscles responsible for the contraction of the rectum by activating the light source surrounding or located near the sacral spinal nerves and/or nerve fibers arising from the inferior hypogastric plexus when the individual experiences the need and/or urge to defecate or wishes to defecate according to a predetermined schedule.

[00152] In other aspects, the method can also comprise electrical stimulation which can be used to depolarize the innervations of the detrusor muscle and/or the pudendal innervations of the external urinary sphincter as well as the innervations of the external anal sphincter. In some embodiments, an externally-controllable electrode is placed around or near the neurons responsible for the innervation of the detrusor muscle, the neurons responsible for the innervation of the external urinary sphincter, and/or the neurons responsible for the innervation of the external anal sphincter. In other embodiments, electrical stimulation can be used to depolarize the detrusor innervations arising from the sacral spinal nerves, resulting in the contraction of the detrusor muscle and the voiding of the urinary bladder while light-responsive opsin proteins can be used to hyperpolarize or cause depolarization-induced synaptic depletion of the detrusor innervations arising from the sacral spinal nerves, resulting in the relaxation of the detrusor muscle, thereby permitting the urinary bladder to fill. In another embodiment, electrical stimulation can be used to depolarize the external urinary sphincter innervations of the pudendal nerve, thereby closing the external urinary sphincter and permitting the bladder to fill while light-responsive opsin proteins can be used to hyperpolarize or cause depolarization-induced synaptic depletion of the external urinary sphincter innervations of the pudendal nerve, resulting in the relaxation of the external urinary sphincter, thereby permitting the urinary bladder to void.

Depolarization with light-responsive opsin proteins for treating sexual dysfunction

[00153] Also provided herein is a method for treating sexual dysfunction in an individual in need thereof, the method comprising administering an effective amount of a polynucleotide comprising a nucleotide sequence encoding a light-responsive opsin protein to the individual, wherein the light-responsive opsin protein is expressed on the plasma membrane of a population of neurons responsible for the innervation of the muscles and organs of the genitalia responsible for sexual function in the individual, wherein the light-responsive opsin protein is capable of causing depolarization of the neurons expressing the light-responsive protein in response to light, whereby the depolarization of the neurons responsible for the innervation of the genitalia permits sexual intercourse and/or sexual functionality in the individual. In some embodiments, sexual functionality is one or more functions selected from the group consisting of erection, orgasm, ejaculation, and

vaginal lubrication. In other embodiments, sexual functionality is restoration of tactile sensations to the genitals needed to maintain sexual arousal. Any of the polynucleotides described herein that encode a light-responsive opsin protein capable of inducing depolarization of neurons may be administered. In other embodiments, the population of neurons can be depolarized to the point of synaptic depletion by exposure to blue, green, yellow, orange, or red light.

[00154] When illuminated by an implantable light source placed around or near the dorsal nerve of the penis/clitoris (a branch of the pudendal nerve) or the sacral spinal nerves, the light-responsive opsin proteins expressed on the plasma membranes of the dorsal nerve of the penis/clitoris cause depolarization of those neurons, leading to the contraction of those muscles and sensations and/or functions associated with normal sexual intercourse. These include, but are not limited to, erection, orgasm, ejaculation, and vaginal lubrication. Additionally, when illuminated by an implantable light source placed around or near the dorsal nerve of the penis/clitoris (a branch of the pudendal nerve) or the sacral spinal nerves, the light-responsive opsin proteins expressed on the plasma membranes of the dorsal nerve of the penis/clitoris cause depolarization of those neurons, leading to the restoration of tactile sensations to the genitals required to maintain sexual arousal.

[00155] In some aspects, the individual externally controls the depolarization of the neurons responsible for the innervation of the genitalia by activating the light means from one or more light sources that surrounds or is located near the pudendal nerve (such as the dorsal nerve of the penis/clitoris branch of the pudendal nerve) or the sacral spinal nerves. In another embodiment, the individual externally changes the polarization state of the neurons responsible for the innervation of the muscles of the genitalia by activating the light means from one or more light sources (such as a light cuff) that surrounds or is located near the pudendal nerve and/or the sacral spinal nerves when the individual experiences the desire to have sexual intercourse or when the individual chooses to have sexual intercourse according to a predetermined schedule. In another embodiment, the individual can be a human.

[00156] In some aspects, the population of neurons responsible for the innervation of the muscles and organs of the genitalia can be transfected by injection of a polynucleotide expression vector (such as any of the polynucleotide expression vector described above) into the somatic motor neuron cell body of a sacral spinal nerve (such as any of S1, S2, S3, S4, and/or S5). In other aspects, population of neurons responsible for the innervation of the muscles of the genitalia can be transfected by injection of the expression vector into the pudendal nerve. In some aspects, one or more light sources (such as a light cuff) capable of generating light in response to an external signal, such as those described above, can be surgically placed around or near the pudendal nerve (such as the dorsal nerve of the penis/clitoris branch of the pudendal nerve) or the sacral spinal nerves.

Kits

[00157] In some aspects, the present invention provides kits for treating a bladder dysfunction, fecal incontinence, and/or sexual dysfunction in an individual in need thereof. In some embodiments, the

kit comprises a polynucleotide comprising a nucleotide sequence encoding a light-responsive ion pump protein, wherein the polynucleotide comprises a nucleotide sequence encoding an amino acid sequence comprising a sequence at least 95% identical to the sequence shown in SEQ ID NO:1, an ER export signal, and a membrane trafficking signal; and a cuff capable of generating light in response to an external signal, wherein the light is capable of activating the light-responsive ion pump protein.

[00158] In some aspects, the present invention provides kits for treating a bladder dysfunction, fecal incontinence, and/or sexual dysfunction in an individual in need thereof. In some embodiments, the kit comprises a polynucleotide comprising a nucleotide sequence encoding a light-responsive ion pump protein, wherein the polynucleotide comprises a nucleotide sequence encoding an amino acid sequence comprising a sequence at least 95% identical to the sequence shown in SEQ ID NO:4, an ER export signal, and a membrane trafficking signal; and a cuff capable of generating light in response to an external signal, wherein the light is capable of activating the light-responsive ion pump protein.

[00159] In some aspects, the present invention provides kits for treating a bladder dysfunction, fecal incontinence, and/or sexual dysfunction in an individual in need thereof. In some embodiments, the kit comprises a polynucleotide comprising a nucleotide sequence encoding a light-responsive ion pump protein, wherein the polynucleotide comprises a nucleotide sequence encoding an amino acid sequence comprising a sequence at least 95% identical to the sequence shown in SEQ ID NO:23, an ER export signal, and a membrane trafficking signal; and a cuff capable of generating light in response to an external signal, wherein the light is capable of activating the light-responsive ion pump protein.

[00160] In other aspects, the kit can further provide a set of instructions for administering an effective amount of the polynucleotide to the individual, wherein the light-responsive ion pump protein is expressed on the plasma membrane of a population of neurons responsible for the innervation of the detrusor muscle, a population of neurons responsible for the innervation of the external urinary sphincter muscle in the individual, and/or a population of neurons responsible for the innervation of the external anal sphincter muscle in the individual; and/or instructions for placing the cuff around the detrusor innervation of the sacral spinal nerves and/or around the external urinary sphincter innervation of the pudendal nerve, and/or around the external anal sphincter innervations of the pudendal nerve or the sacral spinal nerves. The kit can also provide instructions for using any of the light-responsive ion pump proteins described above according to any of the methods described above. Similarly, the light cuffs provided in the kit can be any of the light cuffs described above.

[00161] In some aspects, the present invention provides a kit for treating a bladder dysfunction in an individual in need thereof, where the kit comprises: a) a polynucleotide encoding a light-responsive opsin protein, wherein the polynucleotide comprises a nucleotide sequence encoding an amino acid sequence comprising a sequence at least 95% identical to one or more sequences selected from the

group consisting of SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, and SEQ ID NO:11; and b) a light source capable of generating light in response to an external signal, wherein the light is capable of activating the opsin protein.

[00162] A subject kit can further include instructions for administering an effective amount of the polynucleotide to the individual, where the light-responsive opsin protein encoded by the polynucleotide is expressed on the plasma membrane of a population of neurons responsible for the innervation of the detrusor muscle and/or a population of neurons responsible for the innervation of the external urinary sphincter muscle in the individual; and instructions for placing the light source around the detrusor innervation of the sacral spinal nerve and/or around the external urinary sphincter innervation of the pudendal nerve. In some cases, the bladder dysfunction is detrusor hyperreflexia and/or detrusor-external sphincter dyssynergia.

[00163] In some aspects, the present invention provides a kit for treating fecal incontinence in an individual in need thereof, where the kit comprises: a) a polynucleotide encoding a light-responsive opsin protein, wherein the polynucleotide comprises a nucleotide sequence encoding the opsin protein comprising: i) a sequence at least 95% identical to the sequence shown in SEQ ID NO:1, SEQ ID NO:4, or SEQ ID NO:23; ii) an ER export signal; iii) and a membrane trafficking signal; and b) a light source capable of generating light in response to an external signal, wherein the light is capable of activating the opsin protein.

[00164] A subject kit can further include instructions for administering an effective amount of the polynucleotide to the individual, where the light-responsive opsin protein encoded by the polynucleotide is expressed on the plasma membrane of a population of neurons responsible for the innervation of the external anal sphincter muscle in the individual; and instructions for placing the light source around the external anal sphincter innervations of a sacral spinal nerve and/or the inferior hemorrhoidal branch of the pudendal nerve.

[00165] In some aspects, the present invention provides a kit for treating fecal incontinence in an individual in need thereof, where the kit comprises: a) a polynucleotide encoding a light-responsive opsin protein, where the polynucleotide comprises a nucleotide sequence encoding an amino acid sequence comprising a sequence at least 95% identical to one or more sequences selected from the group consisting of SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, and SEQ ID NO:11; and b) a light source capable of generating light in response to an external signal, wherein the light is capable of activating the opsin protein.

[00166] The kit can further include instructions for administering an effective amount of the polynucleotide to the individual, wherein the light-responsive opsin protein encoded by the polynucleotide is expressed on the plasma membrane of a population of neurons responsible for the innervation of the external anal sphincter muscle in the individual; and instructions for placing the light source around the external anal sphincter innervations of a sacral spinal nerve and/or the inferior hemorrhoidal branch of the pudendal nerve.

[00167] In some aspects, the present invention provides a kit for treating sexual dysfunction in an individual in need thereof, where the kit comprises: a) a polynucleotide encoding a light-responsive opsin protein, wherein the polynucleotide comprises a nucleotide sequence encoding an amino acid sequence comprising a sequence at least 95% identical to one or more sequences selected from the group consisting of SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, and SEQ ID NO:11; and b) a light source capable of generating light in response to an external signal, where the light is capable of activating the opsin protein.

[00168] The kit can further include instructions for administering an effective amount of the polynucleotide to the individual, where the light-responsive opsin protein encoded by the polynucleotide is expressed on the plasma membrane of a population of neurons responsible for the innervation of the genitalia in the individual; and instructions for placing the light source around the genital innervations arising from one or more sacral spinal nerves, the dorsal nerve of the penis branch of the pudendal nerve, and/or the dorsal nerve of the clitoris branch of the pudendal nerve.

EXAMPLES

Example 1: Use of optogenetic-based nerve stimulation in an animal model of detrusor external sphincter dysnergia (DSD) and detrusor hyperreflexia (DH)

[00169] This Example validates an animal model of DSD and DH for treatment with the optogenetic methods described herein. Cat models with spinal cord injuries have been used to recreate the human conditions of DSD and DH, having been validated using PET (Tai et al., 2004, *Experimental Neurol.*, 190:171). There are also animal models of hyperreflexia in spinal cord injured (SCI) rats (Shaker et al., 2003, *Neurourol Urodyn.*, 22(7):693-8) as well as in the EAE mouse, which is also a model for multiple sclerosis (Vignes et al., 2007, *J. Physio.* 578(Pt 2):439-50). In this Example, the membrane-targeted photoactivatable anion pump halorhodopsin from *Natronomonas pharaonis* (NpHR) is used to hyperpolarize the nerves responsible for the innervation of the detrusor muscle of the bladder and the external urinary sphincter.

Materials and Methods

[00170] Cats with experimentally induced spinal cord injuries to replicate symptoms of human DSD and DH are produced according to known methods (Tai et al., 2004, *Experimental Neurol.* 190:171). Yellow fluorescent protein (YFP)-labeled Halorhodopsin (YFP-NpHr3.0, and/or YFP-NpHR3.1; see [www\(dot\)optogenetics\(dot\)org](http://www.optogenetics.org)) in an AAV1 viral vector and under control of the feline *Thy1* promoter is injected directly into the somatic motor neuron cell body of the sacral spinal nerves responsible for detrusor innervations. Additionally, YFP-halorhodopsin in an AAV1 viral vector under control of the feline *Thy1* promoter is injected into Onuf's nucleus, the origin of the pudendal nerve.

Light cuff construction and surgical implantation

[00171] Light cuff construction uses a 0.5 mm interior diameter borosilicate glass pipette. The glass pipettes is carefully ground to 4 mm and an approximately 0.5 mm wide channel is ground

longitudinally along the length of the cuff using a dental drill with an abrasive tip. Ultraviolet-curing epoxy (Norland Products, Cranbury, NJ, NOA 81) is applied to the outside of the cuff and cured with a UV curing lamp. Sixteen small (1.0 x 0.6 x 0.2 mm) amber LEDs (Rohm, Kyoto, Japan, SMLP12BC7T, 589 nm) are applied to the exterior of the glass cuff and secured in place with the UV-cured optical epoxy. LEDs are arranged in a concentric perimeter facing the peripheral nerve in the center and micro-soldered by hand using 40 gauge copper magnet wire. Intensity and on/off times of single LEDs are measured with a digital optical power meter and a high-speed photo-detector (ThorLabs, Newton, NJ, S130A and DET10A). Intensity measurements are used as inputs for a light propagation model, described below, and serve to construct an electrical input current vs. light intensity plot for estimation of experimental light.

[00172] The externally-activatable light cuff with light emitting diode (LED) is surgically placed so as to encircle each nerve (one or more sacral spinal nerve and/or the pudendal nerve; *see, e.g.*, Llewellyn et al., 2010, *Nat. Med.*, 16(10):161-165).

Light propagation model

[00173] A light propagation model is used to verify that all regions of the detrusor innervations arising from the sacral spinal nerves as well as the external urinary sphincter innervations of the pudendal nerve (or the pudendal nerve itself) are exposed to light intensities that exceeded that minimum light intensity required to activate NpHr3.0, and/or NpHR3.1 chloride pumps (3.5 mW/mm²; Gradinaru et al., 2010, *Cell*, 141:1-12). Light propagation is modeled using the Kubelka-Munk model as outlined in Aravanis et al. for diffuse scattering tissue (2007, *J Neural Eng* 4, S143-56). Briefly, the light intensity varies with distance by the equation

$$\frac{I(z)}{I(z=0)} = \frac{\rho^2}{(Sz + 1)(z + \rho)}$$

where

$$\rho = r \sqrt{\left(\frac{n}{NA}\right)^2 - 1}$$

and S was the scattering length of the tissue, z was the distance from the light source, r was the diameter of the LED chip, n was the refractive index of the material the light was traversing, NA was the numerical aperture of the LED from

$$NA = n \sin \theta_{1/2}$$

and $1/2$ was the half angle of divergence from the LED. This model takes into account the effect on light intensity due to scattering and geometrical losses. The value used for scattering length is empirically determined from mouse brain slices for wavelengths between 400 and 900 nm, and assumed to be similar in peripheral tissue. This model also assumes no losses in light intensity due to absorption, nor does it take into account multiply scattering photons. Values for $1/2$ and r are taken

from the LED manufacturer's product sheet, while $I(z=0)$ was measured with a power meter (ThorLabs, Newton, NJ, S130A) for a single LED.

[00174] Using a pudendal nerve diameter as a reference in this model, relative optical intensity variation can be determined on the nerve periphery and at the nerve center. Any drop in intensity across the nerve can therefore be determined as well as the minimum surface of the nerve required to exceed the minimal intensity for light activation of NpHr3.0, and/or NpHR3.1 chloride pumps (3.5 mW/mm^2). The light cuff is capable of exceeding 25 mW mm^{-2} at the surface of the nerve.

Motor axon imaging of the pudendal nerve and sacral spinal nerves

[00175] Adult spinal cord-injured cats, prepared as described above, are anesthetized by injection of ketamine and xylazine. The detrusor muscle and/or external urinary sphincter muscles are exposed by incision in the skin followed by intramuscular injection of $4 \mu\text{l}$ of 5% retrograde labeling dye (Fast Blue, Polysciences, Warrington, PA). The skin incision is closed by a tissue adhesive (VetBond, 3M, St. Paul, MN), and cats are allowed to recover. After one week, animals are anesthetized and sacrificed. Pudendal and sacral spinal nerve sections are dissected and fixed in 4% paraformaldehyde for 30 min at 25°C . Samples are then washed twice in 1X phosphate-buffered saline (in mM, 2.7 KCl, 1.76 KH_2PO_4 , 137 NaCl, 10 NaHPO_4 , pH 7.4) for 5 min each at 25°C , embedded in 50°C lowmelting point agarose, and vibratome sliced into $50 \mu\text{m}$ sections.

[00176] Following a 30 min permeabilization in 0.1% Triton X-100 (octylphenolpoly (ethyleneglycolether)x) and 3% normal donkey serum, sections are incubated overnight with mouse monoclonal anti-lamin 1:500 (Abcam, Cambridge, MA) and rabbit polyclonal anti-tau 1:1000 (DAKO, Cambridgeshire, UK). Sections are then washed and incubated for 3 hrs at 25°C with fluorescent Cy3- or Cy5-conjugated secondary antibodies 1:1000 (Jackson Laboratories, West Grove, PA). Confocal fluorescence images are acquired using a Leica TCS SP5 scanning laser microscope (Leica Microsystems GmbH, Wetzlar, Germany) with a 20X/0.70NA or 40X/1.25NA oil immersion objective. Multiple serial stack images across several subjects are acquired using equivalent settings.

Image analysis

[00177] The number, size, and fluorescence intensity of motor axons ($3 \mu\text{m}$ and G-ratio 0.5) are determined by manual analysis using ImageJ software (NIH, Bethesda, MD). The perimeter of lamin-labeled myelin sheath and tau-labeled axolemma are outlined by hand to form a region of interest. The Feret's diameter and average pixel intensity was then automatically determined in ImageJ for the region of interest. The axon depth within the pudendal and sacral spinal nerves are determined by finding the shortest distance between the center of each axon and the exterior of the nerve. Motor axon diameter vs. pixel intensity of yellow fluorescent protein are tested for statistically significant correlation using a paired two-tailed Student's t -test ($\alpha=0.05$) after first testing for normality using Lilliefors test ($\alpha=0.05$) in Matlab (Mathworks, Natick, MA).

[00178] The distribution of the YFP-tagged light-responsive chloride pumps within motor axons of a *Thyl::NpHR* cat are quantified by examining cross-sections of the pudendal and sacral spinal nerves both parallel and perpendicular to the long axis of the axons.

[00179] Stimuli provided via the light cuffs evoke electrical and contractile responses of the detrusor muscle and the external urinary sphincter.

[00180] An external signal is used to activate the LEDs. The activation of the sacral spinal LEDs and the pudental LEDs are such that when one is on, the other is off. When the sacral spinal LEDs are on, the bladder is relaxed and the external urinary sphincter is active, allowing for the storage of urine. When the sacral spinal LEDs are off, the external urinary sphincter is on allowing the external urinary sphincter to relax and allow the urine to pass from the contracting bladder. When voiding is complete, the sacral spinal LEDs are turned back on with external urinary sphincter LEDs being turned off.

Example 2: Use of light-responsive cation channels to provoke depolarization-induced synaptic depletion in an animal model of detrusor external sphincter dysnergia (DSD) and detrusor hyperreflexia (DH)

[00181] This Example validates an animal model of DSD and DH for treatment with the optogenetic methods described above whereby urinary function is restored via selective depolarization-induced synaptic depletion of the detrusor innervations arising from the sacral spinal nerves and the external urinary sphincter innervations of the pudental nerve. The feline or rodent animal models are identical to those used in Example 1.

[00182] Yellow fluorescent protein (YFP)-labeled SSFO (pAAV-*Thyl*-hChR2 (E123T/T159C)-EYFP; *see* [www\(optogenetics\(dot\)org](http://www.optogenetics.org)) in an AAV1 viral vector and under control of the feline *Thyl* promoter is injected directly into the somatic motor neuron cell body of the sacral spinal nerves (responsible for detrusor innervations) and into Onuf's nucleus (responsible for external urinary sphincter innervations).

[00183] Light cuffs are manufactured as in Example 1. The externally-activatable light cuffs with light emitting diode (LED) are surgically placed so as to encircle the detrusor innervations arising from the sacral spinal nerves and the external urinary sphincter innervations of the pudental nerve. The depolarization-induced synaptic depletion caused by activation of the light-responsive cation channel proteins with light having an intensity of at least 100 Hz evokes the relaxation of the detrusor muscle, thereby permitting the bladder to fill. Additionally, the depolarization-induced synaptic depletion caused by activation of the light-responsive cation channel proteins with light having an intensity of at least 100 Hz causes the relaxation of the external urinary sphincter, thereby permitting voiding of urine.

[00184] An external signal is used to activate the LEDs. The activation of the sacral spinal LEDs and the pudental LEDs are such that when one is on, the other is off. When the sacral spinal LEDs are on, the bladder is relaxed and the external urinary sphincter is active, allowing for the storage of

urine. When the sacral spinal LEDs are off, the external urinary sphincter is on allowing the external urinary sphincter to relax and allow the urine to pass from the contracting bladder. When voiding is complete, the sacral spinal LEDs are turned back on with external urinary sphincter LEDs being turned off.

Example 3: AAV Vector Constructs

[00185] The following Adenoassociated virus (AAV) constructs were generated: 1) AAV1:hsyn-ChR2-EYFP (AAV1 comprising a nucleotide sequence encoding a ChR2-eYFP fusion protein operably linked to a human synapsin 1 promoter); 2) AAV6-hsyn-ChR2-EYFP (AAV6 comprising a nucleotide sequence encoding a ChR2-eYFP fusion protein operably linked to a human synapsin 1 promoter); 3) AAV1-hsyn-NpHR-EYFP (AAV1 comprising a nucleotide sequence encoding an NpHR 3.0-EYFP fusion protein, operably linked to a human synapsin 1 promoter); 4) AAV6-hsyn-NpHR-EYFP (AAV6 comprising a nucleotide sequence encoding an NpHR 3.0-EYFP fusion protein, operably linked to a human synapsin 1 promoter); 5) AAV1-hsyn-eARCH-EYFP (AAV1 comprising a nucleotide sequence encoding an eARCH 3.0-EYFP fusion protein, operably linked to a human synapsin 1 promoter).

[00186] Single-stranded DNA AAV viruses were produced in a baculovirus system (Virovek, Hayward, CA; as described in WO 2008/024998

Example 4: Intramuscular Injections of AAV Encoding Opsins in Rat

[00187] A total dose of about 1×10^{12} viral genomes (vg) in about 12 μ L of constructs described in Example 3 was injected into the EUS muscle of female F344/Sprague Dawley rats, 130-170 g each. At various numbers of days after injection, animals were sacrificed, and expression of the opsins encoded by the constructs in the dorsolateral nucleus (DLN), and other regions of the spinal cord, was assessed. Figure 1 depicts schematically the various sections of the spinal cord, including the DLN. All constructs encoded opsin-EYFP fusion proteins (e.g., ChR2-EYFP; NpHR-EYFP; eARCH-EYFP).

[00188] 40 nm sections of the rat spinal cord were prepared.. Sections were stained with DAPI and examined with a fluorescence microscope. Confocal microscopy was used to count DLN motor neurons on all sections by visualizing YFP labeling. Motor neurons were visualized using a 40x objective, and photomicrographs for sections were taken by merging images from DAPI (depicting the nucleus) and YFP (indicating regions of the cell body) channels. Sections with a visible nucleus surrounded by YFP within the DLN were counted over serial sections to determine total motor neuron counts for each animal. In each section, cells were counted as positive if they showed both YFP expression and DAPI for nuclear staining. The data are depicted in Figure 2.

[00189] As shown in Figure 2, left panel, approximately half of all the pudendal motor neurons expressed protein encoded by the various injected AAV constructs. Literature values for total number of pudendal motor neurons as measured by retrograde labeling is approximately 60 for DL right and DL left, and 120 total. (Kane et al. (2002) *Anat. Rec.* 266:21-29).

Example 5: Measuring Contraction of EUS Muscle and Bladder Pressure in Rat

[00190] 90 days after AAV1-hsyn-ChR2-EYFP intramuscular injection into the EUS, cystometry and sphincter-EMG recordings of the bladder and external urinary sphincter were performed. Cystometry involved placing a catheter in the rodent bladder that is hooked to a pressure sensor. Electrodes were placed in the EUS and hooked up to a recording amplifier to measure sphincter muscle contraction (EMG) activity. The data are presented in Figure 3.

[00191] Figure 3 shows EUS muscle contractions at different frequencies/time durations after blue light optical stimulation at the pudendal nerve of rats intramuscularly (into the EUS) injected with AAV1-hsyn-ChR2-EYFP. Observed optically-induced contractions follow the frequency of light pulses from 5-50 Hz for the pudendal nerve.

[00192] 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.

SEQUENCES

The amino acid sequence of NpHR without the signal peptide:

VTQRELFEFVLNDPLLASSLYINIALAGLSILLFVFMTRGLDDPRAKLIADVSTILVPVVSIASTY
GLASGLTISVLEMPAGHFAEGSSVMLGGEEVDGVVTMWGRYLTWALSTPMILLALGLLAG
SNATKLFTAITFDIAMCVTGLAAALTSSHLMRWFWYAISCACFLVLYILLVEWAQDAKA
AGTADMFNLTLLTVVMWLGYPVWALGVEGIAVLPVGVTSWGYSLDIVAKYIFAFLLLN
YLTSNESVSVSGSILDVPSASGTPADD (SEQ ID NO:1).

The amino acid sequence of eYFP-NpHR3.0:

MTETLPPVTESAVALQAEVTQRELFEFVLNDPLLASSLYINIALAGLSILLFVFMTRGLDDPR
AKLIADVSTILVPVVSIASTYGLASGLTISVLEMPAGHFAEGSSVMLGGEEVDGVVTMWGRYL
TWALSTPMILLALGLLAGSNATKLFTAITFDIAMCVTGLAAALTSSHLMRWFWYAISCACF
LVVLYILLVEWAQDAKAAGTADMFNLTLLTVVMWLGYPVWALGVEGIAVLPVGVTSW
GYSLDIVAKYIFAFLLLNYLTSNESVSVSGSILDVPSASGTPADDAAAKSRITSEGEYIPLDQID
INVVSKGEELFTGVVPILVELDGDVNGHKFSVSGEGEGDATYGKLTLKFICTTGKLPVPWPT
LVTTFGYGLQCFARYPDHMKQHDFFKSAMPEGYVQERTIFFKDDGNYKTRAIEVKFEGDTL
VNRIELKGIDFKEDGNILGHKLEYNYNSHNVYIMADKQKNGIKVNFKIRHNIEDGSVQLADH

YQNTPIGDGPVLLPDNHYLSYQSALSKDPNEKRDHMLLEFVTAAGITLGMDELYKFCYENEV (SEQ ID NO:2).

The amino acid sequence of eYFP-NpHR3.1:

MVTQRELFEFVLNDPLLASSLYINIALAGLSILLFVFMTRGLDDPRAKLIIVSTILVPVVSIASTGLASGLTISVLEMPAGHFAEGSSVMLGGEEVDGVVTMWGRYLTWALSTPMILLALGLLAGSNATKLFTAITFDIAMCVTGLAAALTSSHLMRWFWYAISCACFLVVLVILLVEWAQDAKAAGTADMFTLKLTVVMWLGYPVWALGVEGIAVLPVGVTSWGYSFLDIVAKYIFAFLLNYLTSNESVVSIGSILDVPSASGTPADDAAKSRITSEGEYIPLDQIDINVVSKGEELFTGVVPILEVELDGDVNGHKFSVSGEGEGDATYGKLTCLKFICTTGKLPVPWPVTLVTTFGYGLQCFARYPDHMKQHDFFKSAMPEGYVQERTIFFKDDGNYKTRAEVKFEGDTLVNRIELKGIDFKEDGNILGHKLEYNYNSHNVIYIMADKQKNGIKVNFKIRHNIEDGSVQLADHYQNTPIGDGPVLLPDNHYLSYQSALSKDPNEKRDHMLLEFVTAAGITLGMDELYKFCYENEV (SEQ ID NO:3).

The amino acid sequence of GtR3:

ASSFGKALLEFVFVIFACITLLLGINAAKSKAASRVLPATFVTGASIAFYFSMASGGGWVIAPDCRQLFVARYLDWLITPPLLIDLGLVAGVSRWDIMALCLSDVLMATGAFGSLTVGNVKWVWWFFGMCWFLHIIIFALGKSWAEAAKAKGGDSASVYSKIAGITVITWFCYPVVWVFAEGFGNFSVTFEVLIYGVLDVISKAVFGLILMSGATGYESI (SEQ ID NO:4).

The amino acid sequence of ChR2:

MDYGGALSAVGRELLFVTNPVVVNGSVLVPEDQCYCAGWIESRGTNGAQTASNVLQWLAGFSILLLMFYAYQTWKSTCGWEEIYVCAIEMVKVILEFFFEFKNPSMLYLATGHRVQWLYAEWLLTCPVILIHLSNLTGLSNDYSRRTMGLLVSDIGTIVWGATSAMATGYVKVIFFCGLCYGANTFFHAAKAYIEGYHTVPKGRCRQVVTGMAWLFFVSWGMFPILFILGPEGFGVLSVYGSTVGHTIIDLMSKNCWGLLGHYLRVLIHEHILIHGDIRKTTKLNIGGTEIEVETLVEDEAEAGAVP (SEQ ID NO:5).

The amino acid sequence of SFO:

MDYGGALSAVGRELLFVTNPVVVNGSVLVPEDQCYCAGWIESRGTNGAQTASNVLQWLAGFSILLLMFYAYQTWKSTCGWEEIYVCAIEMVKVILEFFFEFKNPSMLYLATGHRVQWLYAEWLLTSPVILIHLSNLTGLSNDYSRRTMGLLVSDIGTIVWGATSAMATGYVKVIFFCGLCYGANTFFHAAKAYIEGYHTVPKGRCRQVVTGMAWLFFVSWGMFPILFILGPEGFGVLSVYGSTVGHTIIDLMSKNCWGLLGHYLRVLIHEHILIHGDIRKTTKLNIGGTEIEVETLVEDEAEAGAVP (SEQ ID NO:6).

The amino acid sequence of SSFO:

MDYGGALSAVGRELLFVTNPVVVNGSVLVPEDQCYCAGWIESRGTNGAQTASNVLQWLAGFSILLLMFYAYQTWKSTCGWEEIYVCAIEMVKVILEFFFEFKNPSMLYLATGHRVQWLYAEWLLTSPVILIHLSNLTGLSNDYSRRTMGLLVSAIGTIVWGATSAMATGYVKVIFFCGLCYGANTFFHAAKAYIEGYHTVPKGRCRQVVTGMAWLFFVSWGMFPILFILGPEGFGVLSVYGSTVGHTIIDLMSKNCWGLLGHYLRVLIHEHILIHGDIRKTTKLNIGGTEIEVETLVEDEAEAGAVP (SEQ ID NO:7).

The amino acid sequence of C1V1:

MSRRPWLLALALAVALAAGSAGASTGSDATVPVATQDGPDYVFHRAHERMLFQTSYTLNNGSVICIPNNGQCFCLAWLKSNGTNAEKLAANILQWITFALSALCLMFYGYQTWKSTCGWEEIYVATIEMIKFIIIEYFHEFDEPAVIYSSNGNKTVWLRVYAEWLLTCPVLLIHLSNLTGLKDDYSKRTMGLLVSDVGCIVWGATSAMCTGWTKILFFLISLSYGMYYTFHAAKVYIEAFHT

VPKGICRELVRVMAWTFVFAWGMFPVLFLLGTEGFGHISPYGSAIGHSLDLIAKNMWGV
LGNYLRVKIHEHILLYGDIRKKQKITIAGQEMEVELVAEEED (SEQ ID NO:8).

The amino acid sequence of C1V1 (E122T):

MSRRPWLLALALAVALAAGSAGASTGSDATVPVATQDGPDYVFHRAHERMLFQTSYTLEN
NGSVICIPNNGQCFCLAWLKSNGTNAEKLAANILQWITFALSALCLMFYGYQTWKSTCGWE
TIYVATIEMIKFIEYFHEFDEPAVIYSSNGNKTVWLRYAEWLLTCPVLLIHLNLTGLKDDYS
KRTMGLLVSDVGCIVWGATSAMCTGWTKILFFLISLSYGMYYTFHAAKVYIEAFHTVPKGI
CRELVRVMAWTFVFAWGMFPVLFLLGTEGFGHISPYGSAIGHSLDLIAKNMWGVLGNYLR
VKIHEHILLYGDIRKKQKITIAGQEMEVELVAEEED (SEQ ID NO:9).

The amino acid sequence of C1V1 (E162T):

MSRRPWLLALALAVALAAGSAGASTGSDATVPVATQDGPDYVFHRAHERMLFQTSYTLE
NNGSVICIPNNGQCFCLAWLKSNGTNAEKLAANILQWITFALSALCLMFYGYQTWKSTCG
WEEIYVATIEMIKFIEYFHEFDEPAVIYSSNGNKTVWLRYATWLLTCPVLLIHLNLTGLK
DDYSKRTMGLLVSDVGCIVWGATSAMCTGWTKILFFLISLSYGMYYTFHAAKVYIEAFHT
VPKGICRELVRVMAWTFVFAWGMFPVLFLLGTEGFGHISPYGSAIGHSLDLIAKNMWGV
LGNYLRVKIHEHILLYGDIRKKQKITIAGQEMEVELVAEEED (SEQ ID NO:10).

The amino acid sequence of C1V1 (E122T/E162T):

MSRRPWLLALALAVALAAGSAGASTGSDATVPVATQDGPDYVFHRAHERMLFQTSYTLE
NNGSVICIPNNGQCFCLAWLKSNGTNAEKLAANILQWITFALSALCLMFYGYQTWKSTCG
WETIYVATIEMIKFIEYFHEFDEPAVIYSSNGNKTVWLRYATWLLTCPVLLIHLNLTGLK
DDYSKRTMGLLVSDVGCIVWGATSAMCTGWTKILFFLISLSYGMYYTFHAAKVYIEAFHT
VPKGICRELVRVMAWTFVFAWGMFPVLFLLGTEGFGHISPYGSAIGHSLDLIAKNMWGV
LGNYLRVKIHEHILLYGDIRKKQKITIAGQEMEVELVAEEED (SEQ ID NO:11).

The amino acid sequence of eArch:

MDPIALQAGYDLLGDGRPETLWLIGITLLMLIGTFYFLVRGWGVTDKDAEYYAVTILVP
GIAAAYLSMFFGIGLTEVTVGGEMLDIYYARYADWLFTTPLLDDLALLAKVDRVTIGTL
VGVDALMIVTGLIGALSHTAIARYSWWLFSTICMIVVLYFLATSLRSAAKERGPEVASTFN
TLTALVVLVLTAYPILWIIGTEGAGVVGLGIETLLFMVLDVTAKVGFGLLRRAILGDTE
APEPSAGADVSAAD (SEQ ID NO:23).

The amino acid sequence of eArch3.0-EYFP:

MDPIALQAGYDLLGDGRPETLWLIGITLLMLIGTFYFLVRGWGVTDKDAEYYAVTILVP
GIAAAYLSMFFGIGLTEVTVGGEMLDIYYARYADWLFTTPLLDDLALLAKVDRVTIGTL
VGVDALMIVTGLIGALSHTAIARYSWWLFSTICMIVVLYFLATSLRSAAKERGPEVASTFN
TLTALVVLVLTAYPILWIIGTEGAGVVGLGIETLLFMVLDVTAKVGFGLLRRAILGDTE
APEPSAGADVSAADRPVAVSKAAAKSRITSEGEYIPLDQIDINVVSKGEELFTGVVPILVE
LDGDVNGHKFSVSGEGEDATYGKLTCLKFICTTGKLPVPWPTLVTTFGYGLQCFAFYDPH
MKQHDFFKSAMPEGYVQERTIFFKDDGNYKTRAEVKFEGDTLVNRIELKGIDFKEDGNIL
GHKLEYNYSNHNHYIMADKQKNGIKVNFKIRHNIEDGSVQLADHYQQNTPIGDGPVLLPD
NHLYSYQSALS KDPNEKRDHMLLEFVTAAGITLGMDELYKFCYENEV (SEQ ID NO:24).

CLAIMS

We claim:

1. A method for treating a bladder dysfunction in an individual in need thereof, the method comprising:

administering an effective amount of a polynucleotide comprising a nucleotide sequence encoding a light-responsive opsin protein to the individual, wherein the opsin protein is expressed on the plasma membrane of a population of neurons responsible for the innervation of the detrusor muscle and/or a population of neurons responsible for the innervation of the external urinary sphincter muscle in the individual,

wherein the opsin protein induces hyperpolarization of the neurons expressing the opsin protein in response to light, whereby the hyperpolarization of the neurons responsible for the innervation of the detrusor muscle permits storage of urine in the bladder and the hyperpolarization of the neurons responsible for the innervation of the external urinary sphincter muscle permits voiding urine from the bladder,

wherein the polynucleotide comprises a nucleotide sequence encoding a light-responsive opsin protein comprising: i) a sequence at least 95% identical to the sequence shown in SEQ ID NO:1, SEQ ID NO:4, or SEQ ID NO:23; ii) an ER export signal; and iii) a membrane trafficking signal.

2. The method of claim 1, further comprising:

administering an effective amount of a polynucleotide comprising a nucleotide sequence encoding a light-responsive opsin protein to the individual, wherein the opsin protein is expressed on the plasma membrane of a population of neurons responsible for the innervation of the detrusor muscle and/or a population of neurons responsible for the innervation of the external urinary sphincter muscle in the individual,

wherein the opsin protein induces depolarization of the neurons, whereby the depolarization of the neurons responsible for the innervation of the detrusor muscle permits voiding urine from the bladder and the depolarization of the neurons responsible for the innervation of the external urinary sphincter muscle permits storage of urine in the bladder,

wherein the polynucleotide comprises a nucleotide sequence encoding a light-responsive opsin protein comprising a sequence at least 95% identical to the sequence selected from the group consisting of SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, and SEQ ID NO:11.

3. A method for treating a bladder dysfunction in an individual in need thereof, the method comprising:

administering an effective amount of a polynucleotide comprising a nucleotide sequence encoding a light-responsive opsin protein to the individual, wherein the opsin protein is expressed on the plasma membrane of a population of neurons responsible for the innervation of the detrusor

muscle and/or a population of neurons responsible for the innervation of the external urinary sphincter muscle in the individual,

wherein the opsin protein is capable of inducing depolarization-induced synaptic depletion of the neurons expressing the opsin protein in response to light, whereby the depolarization-induced synaptic depletion of the neurons responsible for the innervation of the detrusor muscle permits storage of urine in the bladder and the depolarization-induced synaptic depletion of the neurons responsible for the innervation of the external urinary sphincter muscle permits voiding urine from the bladder,

wherein the polynucleotide comprises a nucleotide sequence encoding a light-responsive opsin protein comprising a sequence at least 95% identical to the sequence selected from the group consisting of SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, and SEQ ID NO:11.

4. A method for treating a bladder dysfunction in an individual in need thereof, the method comprising:

administering an effective amount of a polynucleotide comprising a nucleotide sequence encoding a light-responsive opsin protein to the individual, wherein the opsin protein is expressed on the plasma membrane of a population of neurons responsible for the innervation of the detrusor muscle and/or a population of neurons responsible for the innervation of the external urinary sphincter muscle in the individual,

wherein the opsin protein is capable of inducing depolarization of the neurons, whereby the depolarization of the neurons responsible for the innervation of the detrusor muscle permits voiding urine from the bladder and the depolarization of the neurons responsible for the innervation of the external urinary sphincter muscle permits storage of urine in the bladder,

wherein the polynucleotide comprises a nucleotide sequence encoding a light-responsive opsin protein comprising a sequence at least 95% identical to the sequence selected from the group consisting of SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, and SEQ ID NO:11.

5. The method of any one of claims 1-4, wherein the bladder dysfunction is detrusor hyperreflexia and/or detrusor-external sphincter dyssynergia.

6. The method of any one of claims 1-4, wherein the bladder dysfunction is associated with spinal cord injury or multiple sclerosis.

7. The method of any one of claims 1-4, wherein the population of neurons responsible for the innervation of the detrusor muscle are neurons arising from one or more sacral spinal nerves in the individual.

8. The method of any one of claims 1-4, wherein the population of neurons responsible for the innervation of the external urinary sphincter muscle comprises neurons of the pudendal nerve in the individual.

9. The method of any one of claims 1-8, wherein a light source capable of generating light in response to an external signal is placed around the detrusor innervations arising from the sacral spinal nerves and/or around the external urinary sphincter innervations of the pudendal nerve, wherein the light is capable of activating the light-responsive opsin protein expressed on the plasma membrane of the neurons.

10. The method of claim 9, wherein the light source is capable of generating red, amber, blue, or green light.

11. The method of claim 9, wherein the individual externally controls the polarization state of the neurons responsible for the innervation of the detrusor muscle and/or neurons responsible for the innervation of the external urinary sphincter by activating the light source surrounding the sacral spinal nerve and/or the pudendal nerve.

12. The method of any one of claims 1-4, wherein the polynucleotide is administered by injection of the polynucleotide into the somatic motor neuron cell body of the sacral spinal nerve.

13. The method of any one of claims 1-4, wherein the polynucleotide is administered by injection of the polynucleotide into the pudendal nerve.

14. The method of any one of claims 1-4, wherein the polynucleotide is administered by injection of the polynucleotide into the Onuf's nucleus.

15. A method for treating fecal incontinence in an individual in need thereof, the method comprising:

administering an effective amount of a polynucleotide comprising a nucleotide sequence encoding a light-responsive opsin protein to the individual, wherein the opsin protein is expressed on the plasma membrane of a population of neurons responsible for the innervation of the external anal sphincter muscle in the individual,

wherein the opsin protein induces hyperpolarization of the neurons expressing the opsin protein in response to light, whereby the hyperpolarization of the neurons responsible for the innervation of the external anal sphincter muscle permits defecation,

wherein the polynucleotide comprises a nucleotide sequence encoding a light-responsive opsin protein comprising: i) a sequence at least 95% identical to the sequence shown in SEQ ID NO:1, SEQ ID NO:4, or SEQ ID NO:23; ii) an ER export signal; iii) and a membrane trafficking signal.

16. The method of claim 15, further comprising:

administering an effective amount of a polynucleotide comprising a nucleotide sequence encoding a light-responsive opsin protein to the individual, wherein the opsin protein is expressed on the plasma membrane of a population of neurons responsible for the innervation of the external anal sphincter muscle in the individual,

wherein the opsin protein induces depolarization of the neurons, whereby the depolarization of the neurons responsible for the innervation of the external anal sphincter muscle permits storage of stool in the rectum,

wherein the polynucleotide comprises a nucleotide sequence encoding a light-responsive opsin protein comprising a sequence at least 95% identical to the sequence selected from the group consisting of SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, and SEQ ID NO:11.

17. A method for treating fecal incontinence in an individual in need thereof, the method comprising:

administering an effective amount of a polynucleotide comprising a nucleotide sequence encoding a light-responsive opsin protein to the individual, wherein the opsin protein is expressed on the plasma membrane of a population of neurons responsible for the innervation of the external anal sphincter muscle in the individual,

wherein the opsin protein is capable of inducing depolarization-induced synaptic depletion of the neurons expressing the opsin protein in response to light, whereby the depolarization-induced synaptic depletion of the neurons responsible for the innervation of the external anal sphincter muscle permits defecation,

wherein the polynucleotide comprises a nucleotide sequence encoding a light-responsive opsin protein comprising a sequence at least 95% identical to the sequence selected from the group consisting of SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, and SEQ ID NO:11.

18. A method for treating fecal incontinence in an individual in need thereof, the method comprising:

administering an effective amount of a polynucleotide comprising a nucleotide sequence encoding a light-responsive opsin protein to the individual, wherein the opsin protein is expressed on the plasma membrane of a population of neurons responsible for the innervation of the external anal sphincter muscle in the individual,

wherein the opsin protein is capable of inducing depolarization of the neurons, whereby the depolarization of the neurons responsible for the innervation of the external anal sphincter muscle permits storage of stool in the rectum,

wherein the polynucleotide comprises a nucleotide sequence encoding a light-responsive opsin protein comprising a sequence at least 95% identical to the sequence selected from the group consisting of SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, and SEQ ID NO:11.

19. The method of any one of claims 15-18, further comprising administering an effective amount of a polynucleotide comprising a nucleotide sequence encoding a light-responsive cation channel protein to the individual, wherein the light-responsive cation channel protein is expressed on

the plasma membrane of a population of neurons responsible for the innervation of the muscles responsible for the contraction of the rectum in the individual, wherein the light-responsive cation channel protein is capable of inducing depolarization of the neurons expressing the light-responsive protein in response to light, whereby the depolarization of the neurons responsible for the innervation of the muscles responsible for the contraction of the rectum facilitates defecation.

20. The method of any one of claims 15-19, wherein the fecal incontinence is associated with childbirth, long-term constipation, stroke, diabetes, multiple sclerosis, or spinal cord injury.

21. The method of any one of claims 15-19, wherein the population of neurons responsible for the innervation of the external anal sphincter muscle are neurons arising from one or more sacral spinal nerves in the individual.

22. The method of any one of claims 15-19, wherein the population of neurons responsible for the innervation of the external anal sphincter muscle comprises neurons of the inferior hemorrhoidal branch of the pudendal nerve in the individual.

23. The method of any one of claims 15-19, wherein a light source capable of generating light in response to an external signal is placed around the external anal sphincter innervations of the sacral spinal nerves and/or around the inferior hemorrhoidal branch of the pudendal nerve, wherein the light is capable of activating the light-responsive opsin protein expressed on the plasma membrane of the neurons.

24. The method of claim 23, wherein the light source is capable of generating red, amber, blue, and/or green light.

25. The method of claim 23, wherein the individual externally controls the polarization state of the neurons responsible for the innervation of the external anal sphincter muscle by activating the light source surrounding the external anal sphincter innervations of the sacral spinal nerves and/or around the inferior hemorrhoidal branch of the pudendal nerve.

26. The method of any one of claims 15-19, wherein the polynucleotide is administered by injection of the polynucleotide into the somatic motor neuron cell body of a sacral spinal nerve.

27. The method of any one of claims 15-19, wherein the polynucleotide is administered by injection of the polynucleotide into the pudendal nerve.

28. The method of any one of claims 15-19, wherein the polynucleotide is administered by injection of the polynucleotide into Onuf's nucleus.

29. A method for treating sexual dysfunction in an individual in need thereof, the method comprising:

administering an effective amount of a polynucleotide comprising a nucleotide sequence encoding a light-responsive opsin protein to the individual, wherein the opsin protein is expressed on the plasma membrane of a population of neurons responsible for the innervation of the genitalia of the individual,

wherein the opsin protein induces depolarization of the neurons expressing the opsin protein in response to light, whereby the depolarization of the neurons responsible for the innervation of the genitalia restores sexual function.

30. The method of claim 29, wherein the restored sexual function is one or more functions selected from the group consisting of erection, orgasm, ejaculation, vaginal lubrication, and tactile sensations to the genitals needed to maintain sexual arousal.

31. The method of claim 29, wherein the sexual dysfunction is associated with diabetic neuropathy, multiple sclerosis, tumor, tertiary syphilis, endocrine disorders, hormonal deficiencies, or spinal cord injury.

32. The method of claim 29, wherein the population of neurons responsible for the innervation of the genitalia comprises neurons arising from one or more sacral spinal nerves in the individual.

33. The method of claim 29, wherein the population of neurons responsible for the innervation of the genitalia comprises neurons of the dorsal nerve of the penis branch of the pudendal nerve in a male individual.

34. The method of claim 29, wherein the population of neurons responsible for the innervation of the genitalia comprises neurons of the dorsal nerve of the clitoris branch of the pudendal nerve in a female individual.

35. The method of claim 29, wherein a light source capable of generating light in response to an external signal is placed around the sacral spinal nerves and/or around the innervations of the pudendal nerve, wherein the light is capable of activating the light-responsive opsin protein expressed on the plasma membrane of the neurons.

36. The method of claim 29, wherein the light source is capable of generating red, amber, blue, and/or green light.

37. The method of claim 29, wherein the individual externally controls the polarization state of the neurons responsible for the innervation of the genitalia by activating the light source surrounding the sacral spinal nerve(s) and/or the pudendal nerve.

38. The method of claim 29, wherein the polynucleotide is administered by injection of the polynucleotide into the somatic motor neuron cell body of one or more sacral spinal nerves.

39. The method of claim 29, wherein the polynucleotide is administered by injection of the polynucleotide into the pudendal nerve.

40. The method of claim 29, wherein the polynucleotide is administered by injection of the polynucleotide into Onuf's nucleus.

41. The method of any one of claims 1-40, wherein the polynucleotide is a vector.

42. The method of claim 41, wherein the vector is a viral vector selected from the group consisting of an AAV vector, a retroviral vector, an adenoviral vector, an HSV vector, and a lentiviral vector.

43. The method of claim 42, wherein the viral vector is AAV1, AAV2, AAV6, or AAV9.

44. The method according to claims 1 or 15, wherein the light-responsive opsin protein comprises a signal peptide.
45. The method according to claims 1 or 15, wherein the ER export signal comprises the amino acid sequence FCYENEV (SEQ ID NO:21).
46. The method according to claims 1 or 15, wherein the membrane trafficking signal comprises the amino acid sequence KSRITSEGEYIPLDQIDINV (SEQ ID NO:12).
47. The method of any of claims 1-46, wherein the expression of the light-responsive opsin protein is controlled by a promoter selected from the group consisting of a EF1 α promoter, a CMV promoter, a CAG promoter, a synapsin promoter, a Thy1 promoter, and a CAMKII promoter.
48. A kit for treating a bladder dysfunction in an individual in need thereof, comprising:
- a) a polynucleotide encoding a light-responsive opsin protein, wherein the polynucleotide comprises a nucleotide sequence encoding an opsin protein comprising: i) an amino acid sequence at least 95% identical to the sequence shown in SEQ ID NO:1, SEQ ID NO:4, or SEQ ID NO:23; ii) an ER export signal; iii) and a membrane trafficking signal; and
 - b) a light source capable of generating light in response to an external signal, wherein the light is capable of activating the opsin protein.
49. The kit of claim 48, further comprising instructions for administering an effective amount of the polynucleotide to the individual, wherein the light-responsive opsin protein encoded by the polynucleotide is expressed on the plasma membrane of a population of neurons responsible for the innervation of the detrusor muscle and/or a population of neurons responsible for the innervation of the external urinary sphincter muscle in the individual; and instructions for placing the light source around the detrusor innervation of the sacral spinal nerve and/or around the external urinary sphincter innervation of the pudendal nerve.
50. A kit for treating a bladder dysfunction in an individual in need thereof, comprising:
- a) a polynucleotide encoding a light-responsive opsin protein, wherein the polynucleotide comprises a nucleotide sequence encoding an amino acid sequence comprising a sequence at least 95% identical to one or more sequences selected from the group consisting of SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, and SEQ ID NO:11; and
 - b) a light source capable of generating light in response to an external signal, wherein the light is capable of activating the opsin protein.
51. The kit of claim 50, further comprising instructions for administering an effective amount of the polynucleotide to the individual, wherein the light-responsive opsin protein encoded by the polynucleotide is expressed on the plasma membrane of a population of neurons responsible for the innervation of the detrusor muscle and/or a population of neurons responsible for the innervation of the external urinary sphincter muscle in the individual; and instructions for placing the light source

around the detrusor innervation of the sacral spinal nerve and/or around the external urinary sphincter innervation of the pudendal nerve.

52. The kit of claims 48 or 50, wherein the bladder dysfunction is detrusor hyperreflexia and/or detrusor-external sphincter dyssynergia.

53. A kit for treating fecal incontinence in an individual in need thereof, comprising:

a) a polynucleotide encoding a light-responsive opsin protein, wherein the polynucleotide comprises a nucleotide sequence encoding the opsin protein comprising: i) a sequence at least 95% identical to the sequence shown in SEQ ID NO:1, SEQ ID NO:4, or SEQ ID NO:23; ii) an ER export signal; iii) and a membrane trafficking signal; and

b) a light source capable of generating light in response to an external signal, wherein the light is capable of activating the opsin protein.

54. The kit of claim 53, further comprising instructions for administering an effective amount of the polynucleotide to the individual, wherein the light-responsive opsin protein encoded by the polynucleotide is expressed on the plasma membrane of a population of neurons responsible for the innervation of the external anal sphincter muscle in the individual; and instructions for placing the light source around the external anal sphincter innervations of a sacral spinal nerve and/or the inferior hemorrhoidal branch of the pudendal nerve.

55. A kit for treating fecal incontinence in an individual in need thereof, comprising:

a) a polynucleotide encoding a light-responsive opsin protein, wherein the polynucleotide comprises a nucleotide sequence encoding an amino acid sequence comprising a sequence at least 95% identical to one or more sequences selected from the group consisting of SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, and SEQ ID NO:11; and

b) a light source capable of generating light in response to an external signal, wherein the light is capable of activating the opsin protein.

56. The kit of claim 55, further comprising instructions for administering an effective amount of the polynucleotide to the individual, wherein the light-responsive opsin protein encoded by the polynucleotide is expressed on the plasma membrane of a population of neurons responsible for the innervation of the external anal sphincter muscle in the individual; and instructions for placing the light source around the external anal sphincter innervations of a sacral spinal nerve and/or the inferior hemorrhoidal branch of the pudendal nerve.

57. A kit for treating sexual dysfunction in an individual in need thereof, comprising:

a) a polynucleotide encoding a light-responsive opsin protein, wherein the polynucleotide comprises a nucleotide sequence encoding an amino acid sequence comprising a sequence at least 95% identical to one or more sequences selected from the group consisting of SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, and SEQ ID NO:11; and

b) a light source capable of generating light in response to an external signal, wherein the light is capable of activating the opsin protein.

58. The kit of claim 57, further comprising instructions for administering an effective amount of the polynucleotide to the individual, wherein the light-responsive opsin protein encoded by the polynucleotide is expressed on the plasma membrane of a population of neurons responsible for the innervation of the genitalia in the individual; and instructions for placing the light source around the genital innervations arising from one or more sacral spinal nerves, the dorsal nerve of the penis branch of the pudendal nerve, and/or the dorsal nerve of the clitoris branch of the pudendal nerve.

59. The kit of claims 48, 49, 53, 55, or 57, wherein the amino acid sequence further comprises a signal peptide.

60. The kit of claims 48 or 53, wherein the ER export signal comprises the amino acid sequence FCYENEV.

61. The kit of claims 48 or 53, wherein the membrane trafficking signal comprises the amino acid sequence KSRITSEGEYIPLDQIDINV (SEQ ID NO:12).

62. The kit of any of claims 48-61, wherein the polynucleotide is a vector.

63. The kit of claim 62, wherein the vector is a viral vector selected from the group consisting of an AAV vector, a retroviral vector, an adenoviral vector, an HSV vector, and a lentiviral vector.

64. The kit of claims 48, 49, 53, 55, or 57, wherein the light source is capable of producing amber, red, blue, or green light.

65. The kit of claims 48, 49, 53, 55, or 57, wherein the external signal is a radio signal.

66. The kit of claims 48, 49, 53, 55, or 57, wherein the external signal is a microwave signal.

Figure 1

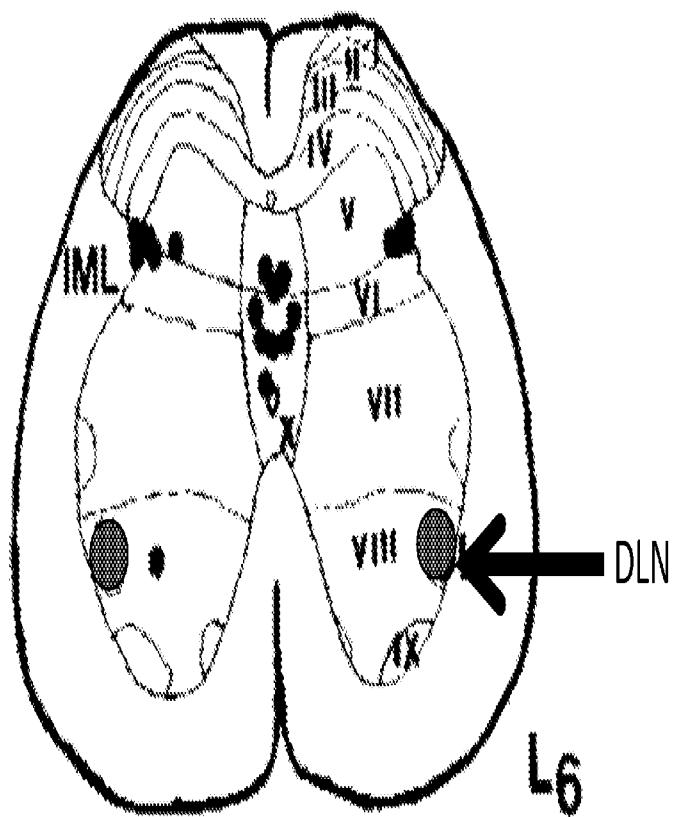


Figure 2
Numbers of EUS Pudendal Motor Neurons: Measured vs. Reported

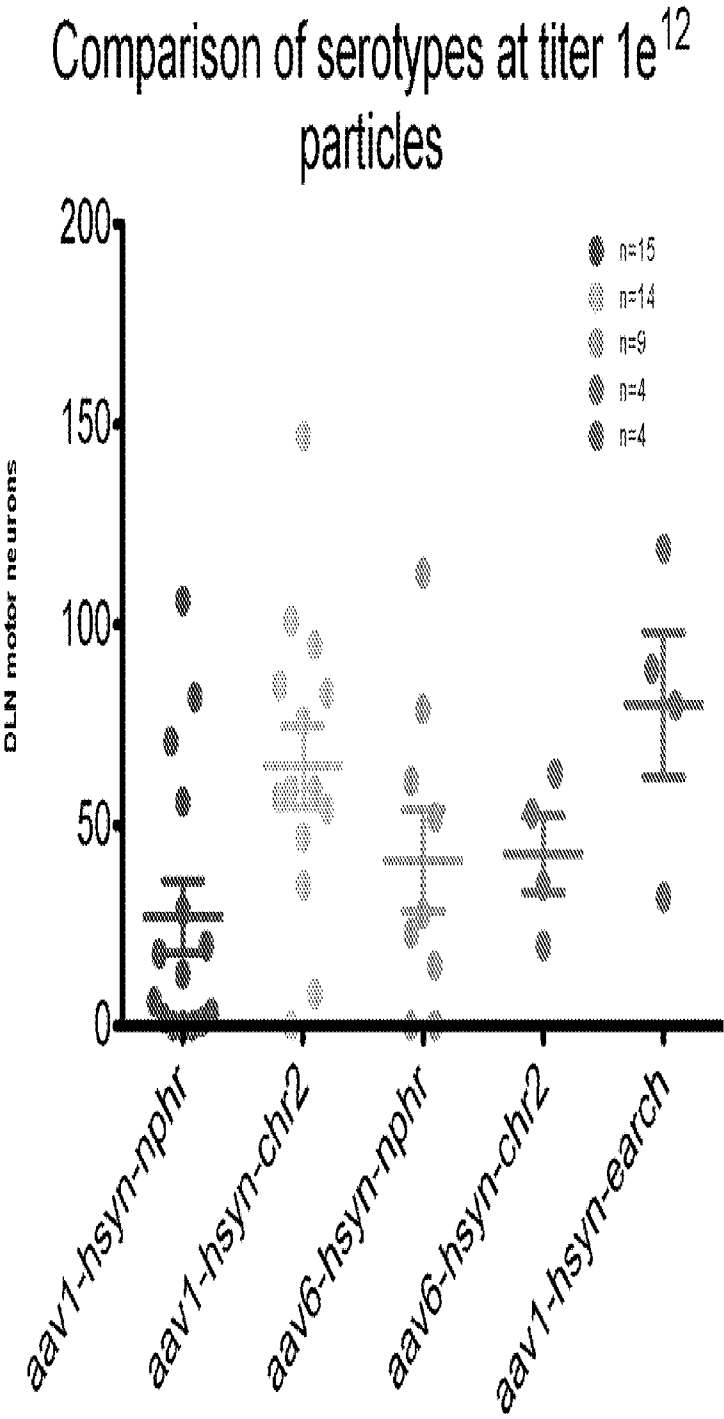


TABLE 1. Pudendal motor neuron counts by retrograde labeling

Location	Mean	±SD	Range	N
Dorsolateral*	60.8	10.6	45.0-71.4	9
DL left	58.0	13.0	33.7-71.5	9
DL right	63.1	11.0	44.1-72.8	9
Dorsomedial*	31.7	8.4	16.5-45.4	9
DM left	31.3	9.1	17.2-43.0	9
DM right	32.1	8.8	14.4-44.6	9
Other*	5.8	12.1	0.0-37.3	9
Other left	1.8	2.9	0.0- 8.7	9
Other right	9.9	24.7	0.0-75.5	9
Breedlove and Arnold, 1980*				
DM	46.9	11.1		5
McKenna and Nadelhaft, 1986*				
DL	57.0	6.6		5
DM	39.5	0.8		3
Oeyama et al., 1987*				
DL	62.7	6.7	52.0-71.0	6
DM	43.6	8.9	28.0-53.0	6
Kerns et al., 2000*				
DL	56.4	23.5		7

*Mean was adjusted by the Abercrombie correction factor.

Kane et al., 2002

Figure 3 Optical Stimulation of Pudendal Nerve Expressing ChR2

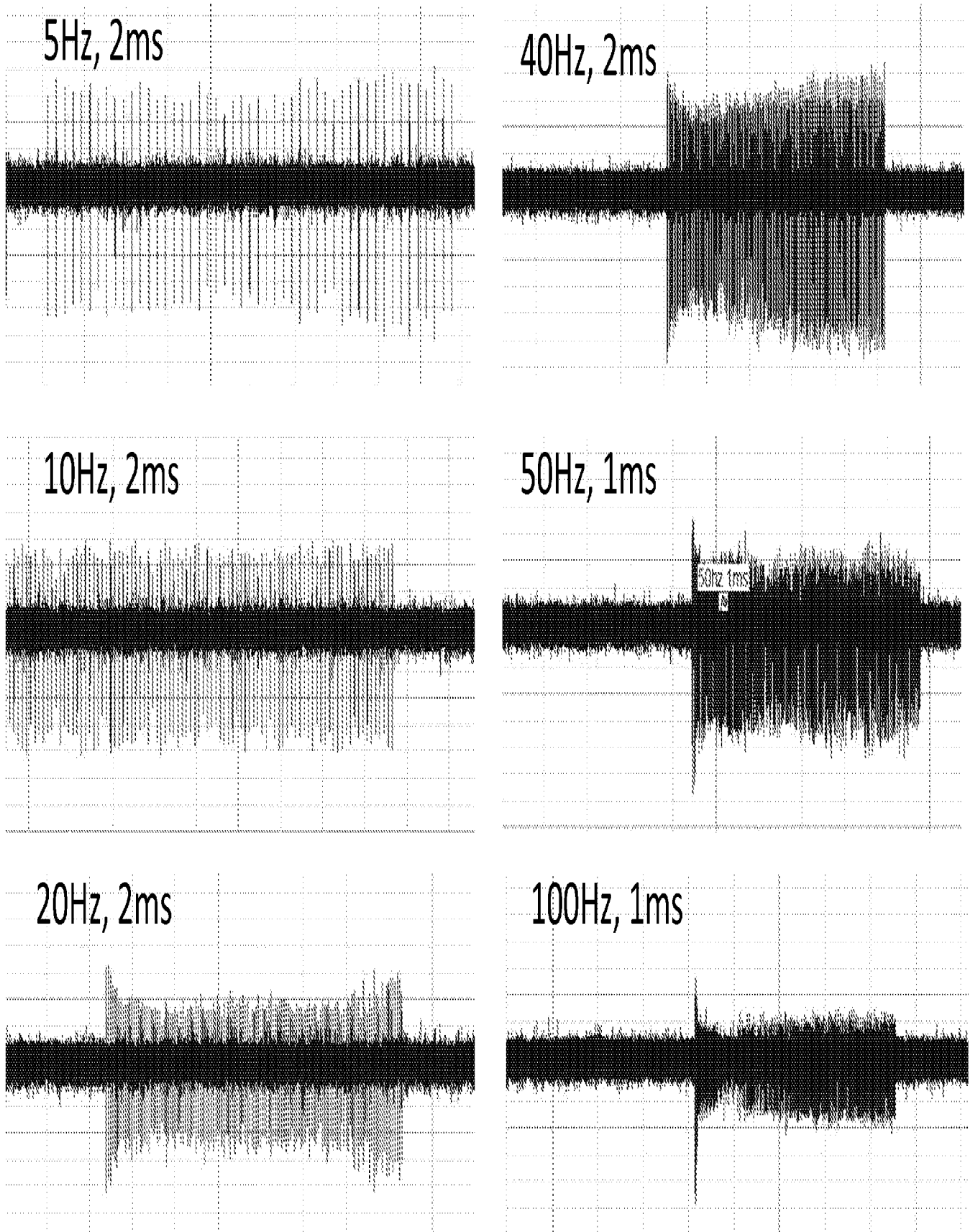
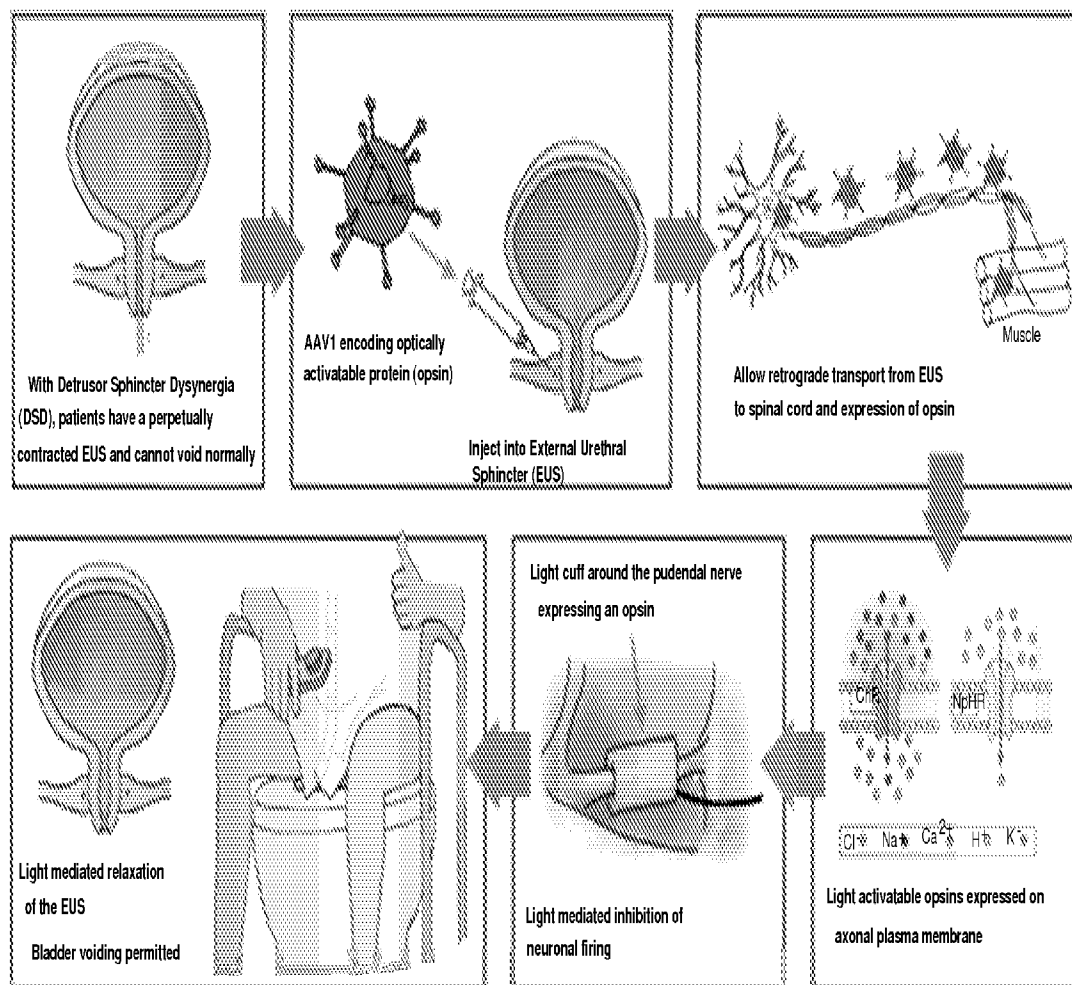


Figure 4

The Problem:

Patient with history of trauma or neurodegenerative disease has a paralyzed bladder



INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2013/027058

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - A61P 21/00 (2013.01)

USPC - 607/88

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC(8) - A61K 48/00; A61P 15/00, 15/10, 15/12, 21/00, 25/00; C07K 14/195, 14/215, 14/405, 14/705; C12N 15/07, 15/63, 15/89 (2013.01)
USPC - 424/422; 514/1.1, 12.1, 17.7, 17.9, 18.1, 21.2, 44R; 530/350; 607/7, 30, 41, 48, 57, 59, 88, 118, 138

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

CPC Class/Subclass(es): A61K 9/0024, 48/00; A61N 1/0524, 1/0556, 1/36007, 5/062 (2013.01)

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Orbit.com, Google Scholar, Google Patents

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2011/116238 A2 (ZHANG et al) 22 September 2011 (22.09.2011) entire document	1-4, 6, 7, 15-19, 29-31, 36, 44-46, 48-51, 53-57, 59-61, 64, 65
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Y		5, 8, 12-14, 32-35, 37-40, 52, 58, 66
Y	US 2009/0326603 A1 (BOGGS et al) 31 December 2009 (31.12.2009) entire document	5, 8, 13, 32-35, 37, 39, 52, 58
Y	US 2009/0069261 A1 (DODGE et al) 12 March 2009 (12.03.2009) entire document	12, 38
Y	US 2007/0253995 A1 (HILDEBRAND) 01 November 2007 (01.11.2007) entire document	14, 40
Y	US 2008/0103551 A1 (MASOUD et al) 01 May 2008 (01.05.2008) entire document	66
A	US 2011/0311489 A1 (DEISSEROTH et al) 22 December 2011 (22.12.2011) entire document	1-8, 12-19, 29-40, 44-46, 48-61, 64-66
T	TYE et al. "Optogenetic investigation of neural circuits underlying brain disease in animal models," Nature Reviews Neuroscience, 20 March 2012 (20.03.2012), Vol. 13, No. 4, Pgs. 251-266. entire document	1-8, 12-19, 29-40, 44-46, 48-61, 64-66

☐ Further documents are listed in the continuation of Box C. ☐

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

21 April 2013

Date of mailing of the international search report

13 MAY 2013

Name and mailing address of the ISA/US

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PCT OSP: 571-272-7774

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2013/027058

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing filed or furnished:

a. (means)

☐

on paper

☒

in electronic form

b. (time)

☒

in the international application as filed

☐

together with the international application in electronic form

☐

subsequently to this Authority for the purposes of search

2. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

SEQ ID NOs: 1-12, 21, and 23 were searched.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2013/027058

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☒ Claims Nos.: 9-11, 20-28, 41-43, 47, 62, 63
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.