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ILSE S. PIENAAR ET AL: "Pharmacogenetic stimulation of cholinergic pedunculopontine neurons reverses motor deficits in a rat model of Parkinson's disease", MOL NEURODEGEN, vol. 10, no. 1, 23 September 2015, DOI: 10.1186/s13024-015-0044-5

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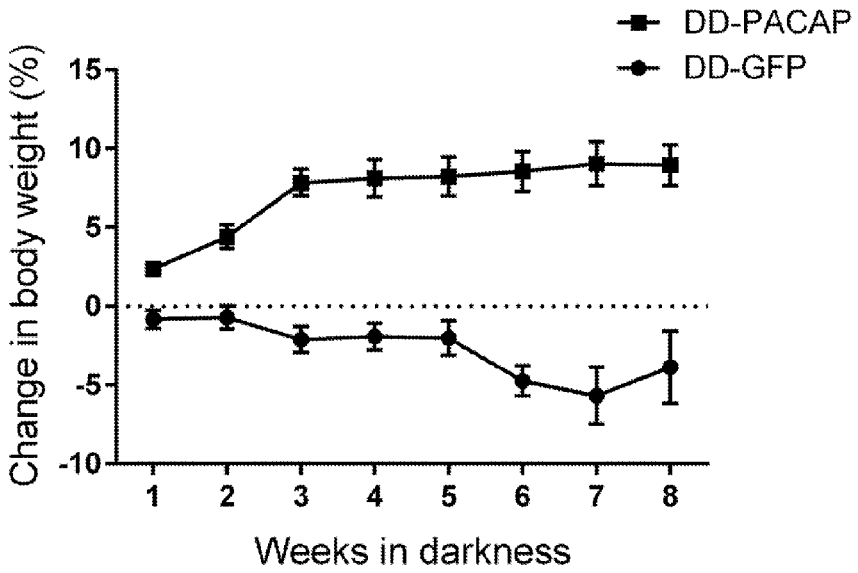
(54) Title: METHODS AND COMPOSITIONS FOR TREATING DISEASES AND DISORDERS OF THE NERVOUS SYSTEM

Figure 6



(57) Abstract: Methods and compositions for treating diseases or disorders of the nervous system using promoter-driven Designer Receptor Exclusively Activated by Designer Drugs (DREADDs) and DREADD agonists are disclosed.

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METHODS AND COMPOSITIONS FOR TREATING DISEASES AND DISORDERS OF THE NERVOUS SYSTEM

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims priority to U.S. Provisional Patent Application Ser. No.: 62/381,883, filed August 31, 2016, which is incorporated herein by reference in its entirety.

SEQUENCE LISTING

The instant application contains a Sequence Listing which has been submitted electronically in ASCII format and is hereby incorporated by reference in its entirety. Said ASCII copy, created on August 31, 2017, is named, "10491-006090-WO0_Sequence_Listing_Final.txt" and is 48,106 bytes in size.

FIELD OF THE INVENTION

The present invention relates to the field of diseases and disorders of the nervous system. Methods and compositions for treating diseases or disorders of the nervous system using promoter-driven Designer Receptor Exclusively Activated by Designer Drugs (DREADDs) and DREADD agonists are disclosed.

BACKGROUND OF THE INVENTION

Diseases and disorders of the nervous system create a significant burden of morbidity and mortality worldwide. Therapeutic treatments are often ineffective because they lack cell-type specificity, even when combined with psychological intervention. Therefore, improved and effective therapeutic treatments are highly desired.

The present invention uses a cell-specific approach to treat diseases and disorders of the nervous system. It was found that neurological pathways associated with photic regulation can be controlled through the retina by leveraging Designer Receptors Exclusively Activated by Designer Drugs (DREADDs). The present invention provides a therapeutic treatment by targeting retinal cells for DREADD expression and circumventing neurosurgical problems associated with injecting DREADDs into the brain. The present invention also allows for stimulation of targeted neurological pathways, especially the brain nucleus locus coeruleus, by natural circuit inputs to provide manipulations of the locus coeruleus in a more physiologically

natural manner, allowing a more clinically applicable treatment than occurs by direct stimulation of locus coeruleus neurons.

SUMMARY OF THE INVENTION

The present invention includes a method of treating a disease or disorder of the nervous system in a subject, comprising the steps of administering an effective amount of a viral vector to the eye of the subject, wherein the viral vector comprises a promoter, a DREADD, and a 3' untranslated region encoded by the nucleotide sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, 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; expressing the DREADD prior to administration of an agonist to the DREADD; and administering to the subject an agonist to the expressed DREADD.

In one embodiment, the viral vector may be an adeno-associated viral vector (AAV) selected from the group consisting of: AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV11, AAV12, and hybrids thereof.

In a second embodiment, the viral vector can be administered intraocularly, intravitreally, sub-retinally, through the sub-internal limiting membrane or by other means know in the art to the eye of the subject.

In another embodiment, the agonist may be clozapine N-oxide, DREADD agonist 21, salvinorin B, clozapine, olanzapine, or perlapine. The agonist may be administered systemically or to the eye.

In another embodiment, the disease or disorder of the nervous system to be treated may be a neuropsychiatric disorder or a neurodegenerative disease. The neuropsychiatric disorder to be treated may be depression, seasonal affective disorder, anxiety, sleep and circadian disorders including desynchronosis, stress disorders including Post Traumatic Stress Disorder (PTSD), Attention Deficit Hyperactivity Disorder (ADHD), autism, addiction, epilepsy, or Intensive Care Unit (ICU) psychosis. The neurodegenerative disease to be treated may be amyotrophic lateral sclerosis (ALS), Parkinson's disease, Alzheimer's disease, or Huntington's disease.

In another embodiment, the disease or disorder of the nervous system is a cerebrovascular accident (CVA) or stroke.

In another embodiment, the method of the present invention may additionally include administering at least one additional therapeutic agent for treatment of the neuropsychiatric disorder or the neurodegenerative disease.

In another embodiment, the at least one additional therapeutic agent for treatment of the neuropsychiatric disorder or the neurodegenerative disease is a neurological drug selected from the group consisting of acamprosate, agomelatine, alimemazine, alprazolam, amantadine, amfetamine, amisulpride, amitriptyline, amobarbital, amoxapine, apomorphine, apomorphine, aripiprazole, asenapine, atomoxetine, atropine, baclofen, benperidol, benztropine, biperiden, bromazepam, bromocriptine, bromperidol, brotizolam, buprenorphine, bupropion, buspirone, butobarbital, cabergoline, carbamazepine, chloral hydrate, chlordiazepoxide, chlorpheniramine, chlorpromazine, chlorprothixene, citalopram, clobazam, clomethiazole, clomipramine, clonazepam, clonidine, clorazepate, clozapine, cyclobarbital, cyproheptadine, cytisine, desipramine, desvenlafaxine, dexamfetamine, dexmethylphenidate, dextromethorphan, diazepam, dicyclomine dimenhydrinate, diphenhydramine, disulfiram, divalproex sodium, donepezil, doxacurium, doxepin, doxylamine, duloxetine, edaravone, enanthate, escitalopram, estazolam, eszopiclone, ethosuximide, flunitrazepam, fluoxetine, flupenthixol, fluphenazine, flurazepam, fluspirilen, fluvoxamine, gabapentin, galantamine, glutethimide, glycopyrrolate, guanfacine, haloperidol, hexamethonium, hydrochloride, hydroxyzine, iloperidone, imipramine, ipratropium, lamotrigine, levetiracetam, levodopa, levomepromazine, levomilnacipran, lisdexamfetamine, lisuride, lithium salts, loprazolam, lorazepam, lormetazepam, mecamylamine, melatonin, melperone, memantine, meprobamate, metamfetamine, methadone, methylphenidate, mianserin, midazolam, mirtazapine, moclobemide, modafinil, modecate, motherwort, nalmefene, naltrexone, niaprazine, nimetazepam, nitrazepam, nortriptyline, olanzapine, omca, ondansetron, orphenadrine, oxazepam, oxcarbazepine, oxitropium, oxybutynin, paliperidone, paroxetine, penfluridol, pentobarbital, perazine, pergolide, pericyazine, perphenazine, phenazepam, phenelzine phenobarbital, phenytoin, pimozide, piribedil, pramipexole, pregabalin, prolixin decanoate, promethazine, propantheline bromide, prothipendyl, protriptyline, quazepam, quetiapine, ramelteon, rasagiline, reboxetine, remacemide, reserpine, riluzole, risperidone, rivastigmine, ropinirole, rotigotine, rubidium chloride, safinamide, scopolamine, secobarbital, sediten, selecten, selegiline, selegiline, sertindole, sertraline, sertraline, sevinol, sinqualone enantat, siqualone, sirtal, sodium oxybate, sodium valproate, solifenacin, stazepine, stelazine,

sulpiride, suvorexant, tacrine, tegretol, telesmin, temazepam, terfluzine, tetrabenazine, thioridazine, thiothixene, tianeptine, timonil, tiotropium, tizanidine, tofisopam, tolcapone, tolterodine, topiramate, trancin, tranylcypromine, trazodone, triazolam, trifluoperaz, trifluoperazine, triftazin, trihexyphenidyl, trimipramine, tropicamide, tubocurarine, valerian, valproate, valproic acid, varenicline, venlafaxine, vilazodone, vortioxetine, zaleplon, ziprasidone, zolpidem, zopiclone, zotepine, zuclopenthixol, and combinations thereof.

The present invention also includes an isolated nucleic acid promoter comprising SEQ ID NO:5, a fragment of SEQ ID NO:5, or a variant of SEQ ID NO: 5 having at least about 75% identity to SEQ ID NO: 5, that retains promoter activity in retinal cells.

The present invention also includes the use of a viral vector comprising a promoter, a DREADD, and a 3' untranslated region encoded by the nucleotide sequences SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, or SEQ ID NO:11 for the treatment of a a disease or disorder of the nervous system in a subject.

In one embodiment, the disease or disorder of the nervous system to be treated by the use of the viral vector may be a neuropsychiatric disorder or a neurodegenerative disease. The neuropsychiatric disorder to be treated may be depression, seasonal affective disorder, anxiety, sleep and circadian disorders including desynchronosis, stress disorders including Post Traumatic Stress Disorder (PTSD), Attention Deficit Hyperactivity Disorder (ADHD), autism, addiction, epilepsy, or Intensive Care Unit (ICU) psychosis.

In a second embodiment, the neurodegenerative disease to be treated by the use of the viral vector may be amyotrophic lateral sclerosis (ALS), Parkinson's disease, Alzheimer's disease, or Huntington's disease.

In another embodiment, the disease or disorder of the nervous system to be treated by the use of the viral vector is a cerebrovascular accident (CVA) or stroke.

The present invention also includes a kit comprising a viral vector, wherein the viral vector comprises a promoter, a DREADD, and a 3' untranslated region encoded by the nucleotide sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, 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 includes an agonist to the DREADD.

BRIEF DESCRIPTIONS OF THE DRAWINGS

Figure 1 provides a graph of the average weight change of rats expressing an excitatory DREADD (G(q)) or GFP (control) in the retina after the indicated number of days in darkness (n=7). An * indicates $p < 0.05$.

Figure 2 shows DREADD expression and function in retinal cells via intravitreal injection (IVI). Figure 2A shows expression of DREADD (arrows) in flat mounted retina. Figure 2B shows expression of DREADD-terminal transport in suprachiasmatic nucleus (arrows). Figure 2C shows CNO administered by intraperitoneal injection (i.p) reduced the size of the amplitude of the electroretinogram (ERG) in animals expressing the inhibitory hM4Di DREADD in retina. Figure 2D shows CNO administered by CNO-containing eye drops similarly decreased the amplitude of the electroretinogram (ERG).

Figure 3 shows DREADD receptor-mediated activation of retinal cells preventing the development of light deprivation-induced depression-like behavior. Figure 3A shows hM3Dq-hSyn activation prevents the relative weight loss that was induced by constant dark (DD) lighting conditions. Figure 3B shows DREADD receptor-mediated activation of retinal cells leads to a reduction in an hedonic-like behavior, as measured by the saccharin preference test. Figure 3C shows a reduction of depression-like behavior as measured by the forced swim test (FST) (C). Figure 3D shows DD-hM3Dq-hSyn activation did not affect behavior typically interpreted as anxiety-like. There is no effect with respect to fecal boli count during the forced swim test. Figure 3E shows no effect with respect to behaviors measured during the elevated plus maze (EPM) assay. Figure 3F shows no effect with respect to locomotor activity. Abbreviations: chronic dark rearing (DD); chronic dark rearing with a control virus expressed in retinal cells (DD-GFP); DREADD receptor-mediated activation of retinal cells during chronic darkness (DD-hM3Dq).

Figure 4 shows that DREADD-mediated activation of retinal cells in constant darkness prevents apoptosis in locus coeruleus. Figure 4A shows that chronic dark rearing (DD-Control) leads to apoptosis in locus coeruleus. Figure 4B shows chronic dark rearing with a control virus expressed in retinal cells (DD-GFP) leads to apoptosis in locus coeruleus. Apoptosis is measured using the *in situ* marker of apoptosis recognizing the p85 fragment of PARP (arrows in Figure 4A and Figure 4B) within the locus coeruleus (shaded, gray). Figure 4C shows that using an hSyn promoter, DREADD receptor-mediated activation of retinal cells during chronic

darkness (DD-hM3Dq) prevents apoptosis in locus coeruleus to a level similar to control animals raised in standard dark/light conditions (DL-Control - Figure 4D). There is arbitrary fluorescence intensity measured using the *in situ* marker of apoptosis recognizing the p85 fragment of PARP within the locus coeruleus boundary (arrows in Figure 4D). Figure 4E shows a comparison of the fluorescence intensity measurements of DD-Control, DD-GFP, DD-hM3Dq and DL-Control.

Figure 5 shows PACAP promoter-driven DREADD expression in melanopsin (+) cells following intravitreal injection (IVI). Arrows show PACAP-hM3Dq positive cells immunoreactive for melanopsin.

Figure 6 shows DREADD activation of PACAP cells driven by a PACAP-specific promoter prevents depression-associated weight loss. Abbreviations: chronic dark rearing with a control virus expressed in retinal cells (DD-GFP); DREADD receptor-mediated activation of PACAP cells driven by the specific PACAP promoter during chronic darkness (DD-PACAP).

DETAILED DESCRIPTION OF THE INVENTION

Definitions

So that the invention may be more readily understood, certain technical and scientific terms are specifically defined below. Unless specifically defined elsewhere in this document, all other technical and scientific terms used have the meaning commonly understood by one of ordinary skill in the art to which this invention belongs.

"Activation," "stimulation," and "treatment," as it applies to cells or to receptors, may have the same meaning, e.g., activation, stimulation, or treatment of a cell or receptor with a ligand, unless indicated otherwise by the context or explicitly.

"Ligand" encompasses natural and synthetic ligands, e.g., cytokines, cytokine variants, analogues, muteins, and binding compounds derived from antibodies. "Ligand" also encompasses small molecules, e.g., peptide mimetics of cytokines and peptide mimetics of antibodies. "Activation" can refer to cell activation as regulated by internal mechanisms as well as by external or environmental factors. "Response," e.g., of a cell, tissue, organ, or organism, encompasses a change in biochemical or physiological behavior, e.g., concentration, density, adhesion, or migration within a biological compartment, rate of gene expression, or state of

differentiation, where the change is correlated with activation, stimulation, or treatment, or with internal mechanisms such as genetic programming.

"Activity" of a molecule may describe or refer to the binding of the molecule to a ligand or to a receptor, to catalytic activity; to the ability to stimulate gene expression or cell signaling, differentiation, or maturation; to antigenic activity, to the modulation of activities of other molecules, and the like. "Activity" of a molecule may also refer to activity in modulating or maintaining cell-to-cell interactions, e.g., adhesion, or activity in maintaining a structure of a cell, e.g., cell membranes or cytoskeleton.

"Administration" and "treatment," as it applies to a human, veterinary, or research subject, refers to therapeutic treatment, prophylactic or preventative measures, to research and diagnostic applications. "Treatment" as it applies to a human, veterinary, or research subject, or cell, tissue, or organ, may encompass the transfection of any of the targeted AAV viral vectors, delivery of promoter-DREADD constructs, or the similar compositions described which are applied to a human or animal subject, a cell, tissue, physiological compartment, or physiological fluid.

"Administration" and "treatment" can refer, e.g., to therapeutic, pharmacokinetic, diagnostic, research, and experimental methods. Treatment of a cell encompasses contact of a reagent to the cell, as well as contact of a reagent to a fluid, where the fluid is in contact with the cell.

"Administration" and "treatment" may also mean *in vitro* and *ex vivo* treatments, e.g., of a cell, by a reagent, diagnostic, binding compound, or by another cell.

"Treat" or "treating" refers to administering a therapeutic agent, such as a composition containing any of the AAV viral vectors, delivery of a promoter-DREADD construct, or similar compositions described, internally or externally to a subject or patient having one or more nervous system disease or disorder symptoms, or being suspected of having a nervous system disease or disorder or being at elevated risk of acquiring a nervous system disease or disorder, or for one or more of another disorder described, i.e., neurodegenerative disorder, for which the agent has therapeutic activity.

The term "prevent" refers to the prophylactic treatment of a subject who is at risk of developing a condition, e.g., a nervous system disease or disorder, resulting in a decrease in the probability that the subject will develop the condition.

The terms "subject" and "patient" are used interchangeably herein. The terms "subject" and subjects" refer to an animal, such as a mammal including a non-primate (e.g. a cow, pig, horse, cat, dog, rat, and mouse) and a primate (e.g. a monkey such as a cynomolgous monkey, a chimpanzee and a human), and for example, a human.

Typically, the agent is administered in an amount effective to alleviate one or more nervous system disease or disorder symptoms in the treated subject or population, whether by inducing the regression of or inhibiting the progression of such symptom(s) by any clinically measurable degree. The amount of a therapeutic agent that is effective to alleviate any particular nervous system disease or disorder symptom (also referred to as the "therapeutically effective amount") may vary according to factors such as the disease state, age, and weight of the patient, and the ability of the drug to elicit a desired response in the subject Whether a nervous system disease or disorder symptom has been alleviated can be assessed by any clinical measurement typically used by physicians or other skilled healthcare providers to assess the severity or progression status of that symptom. While an embodiment of the present invention (e.g., a treatment method or article of manufacture) may not be effective in alleviating the target nervous system disease or disorder symptom(s) in every subject, it should alleviate the target nervous system disease or disorder symptom(s) in a statistically significant number of subjects as determined by any statistical test known in the art such as the Student's t-test, the chi^2-test, the U-test according to Mann and Whitney, the Kruskal-Wallis test (H-test), Jonckheere-Terpstra-test and the Wilcoxon-test.

"Isolated nucleic acid molecule" means DNA or RNA of genomic, mRNA, cDNA, or synthetic origin or some combination thereof, which is not associated with all or a portion of a polynucleotide in which the isolated polynucleotide is found in nature.

For purposes of this disclosure, it should be understood that "a nucleic acid molecule comprising" a particular nucleotide sequence does not encompass intact chromosomes. Isolated nucleic acid molecules "comprising" specified nucleic acid sequences may include, in addition to the specified sequences, coding sequences for up to ten or up to twenty or more other proteins or portions or fragments thereof, or may include operably linked regulatory sequences that control expression of the coding region of the recited nucleic acid sequences, and/or may include vector sequences.

The phrase "control sequences" refers to DNA sequences necessary for the expression of an operably linked coding sequence in a particular host organism. The control sequences that are suitable for prokaryotes, for example, include a promoter, optionally an operator sequence, and a ribosome binding site. Eukaryotic cells are known to use promoters, polyadenylation signals, and enhancers.

A nucleic acid is "operably linked" when it is placed into a functional relationship with another nucleic acid sequence. For example, DNA for a pre-sequence or secretory leader is operably linked to DNA for a polypeptide if it is expressed as a preprotein that participates in the secretion of the polypeptide; a promoter or enhancer is operably linked to a coding sequence if it affects the transcription of the sequence; or a ribosome binding site is operably linked to a coding sequence if it is positioned so as to facilitate translation. Generally, "operably linked" means that the DNA sequences being linked are contiguous, and, in the case of a secretory leader, contiguous and in reading phase. However, enhancers do not have to be contiguous. Linking is accomplished by ligation at convenient restriction sites. If such sites do not exist, the synthetic oligonucleotide adaptors or linkers are used in accordance with conventional practice.

As used, the terms "recombinant cell" refers to a cell into which an exogenous DNA segment, such as DNA segment that leads to the transcription of a biologically-active polypeptide or production of a biologically active nucleic acid such as an mRNA, has been introduced.

The term "vector" includes any genetic element, such as a plasmid, phage, transposon, cosmid, chromosome, artificial chromosome, virus, virion, etc., which is capable of replication when associated with the proper control elements and which can transfer gene sequences between cells. Thus, the term includes cloning and expression vehicles, as well as viral vectors. In some embodiments, useful vectors are contemplated to be those vectors in which the nucleic acid segment to be transcribed is positioned under the transcriptional control of a promoter.

A "promoter" refers to a DNA sequence recognized by the synthetic machinery of the cell, or introduced synthetic machinery, required to initiate the specific transcription of a gene. The phrases "operatively positioned," “operatively linked,” "under control," or "under transcriptional control" means that the promoter is in the correct location and orientation in relation to the nucleic acid to control RNA polymerase initiation and expression of the gene. The term "expression vector or construct" means any type of genetic construct containing a nucleic

acid in which part or all of the nucleic acid encoding sequence is capable of being transcribed. In some embodiments, expression includes transcription of the nucleic acid, for example, to generate a biologically-active polypeptide product or inhibitory RNA (e.g., shRNA, miRNA) from a transcribed gene.

The term "agonist" refers to an agent, e.g., ligand, protein, polypeptide, peptide, lipid, antibody, antibody fragment, large molecule, or small molecule that binds to a receptor and has an intrinsic effect such as inducing a receptor-mediated response. For example, the agonist may stimulate, increase, activate, facilitate, enhance, or up regulate the activity of the receptor. In a particular embodiment, the agonist is a ligand.

"Pharmaceutically acceptable" indicates approval by a regulatory agency of the Federal or a state government or listed in the U.S. Pharmacopeia or other generally recognized pharmacopeia for use in animals, and more particularly in humans.

A "carrier" refers to, for example, a diluent, adjuvant, preservative (e.g., Thimerosal, benzyl alcohol), anti-oxidant (e.g., ascorbic acid, sodium metabisulfite), solubilizer (e.g., polysorbate 80), emulsifier, buffer (e.g., Tris HCl, acetate, phosphate), antimicrobial, bulking substance (e.g., lactose, mannitol), excipient, auxiliary agent or vehicle with which an active agent of the present invention is administered. Pharmaceutically acceptable carriers can be sterile liquids, such as water and oils, including those of petroleum, animal, vegetable or synthetic origin. Water or aqueous saline solutions and aqueous dextrose and glycerol solutions may be employed as carriers, particularly for injectable solutions. Suitable pharmaceutical carriers are described in "Remington's Pharmaceutical Sciences" by E.W. Martin (Mack Publishing Co., Easton, PA); Gennaro, A. R., Remington: The Science and Practice of Pharmacy, (Lippincott, Williams and Wilkins); Liberman, et al., Eds., Pharmaceutical Dosage Forms, Marcel Decker, New York, N.Y.; and Kibbe, et al., Eds., Handbook of Pharmaceutical Excipients, American Pharmaceutical Association, Washington.

A "therapeutically effective amount" of a compound or a pharmaceutical composition refers to an amount effective to prevent, inhibit, or treat a particular disorder or disease and/or the symptoms thereof. For example, "therapeutically effective amount" may refer to an amount sufficient to modulate the nervous system disease or disorder in a subject.

Nucleic Acids

The present invention also comprises certain constructs and nucleic acids encoding DREADD sequences. These constructs and sequences include promoter-DREADD sequences, i.e., PACAP-hM3D(Gq)-mCherry (SEQ ID: 1), TAC-1-hM4D(Gi)-mCherry (SEQ ID NO:2), PRSx8-HA-hM3D(Gq) (SEQ ID NO:3), PRSx8-HA-hM4D(Gi) (SEQ ID NO:4), PACAP-hM3D(Gq) (SEQ ID NO:6), PRSx8-hM3D(Gq) (SEQ ID NO:7), PRSx8-hM4D(Gi) (SEQ ID NO:8), TAC-1-hM4D(Gi) (SEQ ID NO:9), TAC-1-hM3D(Gq)-mCherry (SEQ ID NO:10) and PACAP-hM4D(Gi)-mCherry (SEQ ID NO:11), which can be useful in certain embodiments.

In some embodiments, constructs and nucleic acids encoding DREADD sequences comprise fluorophores, e.g., mCherry in SEQ ID NO:1. The expression of a DREADD can be successfully detected if it is tagged with a fluorescent marker, e.g., GFP, tdTomato, or mCherry.

Included also is an isolated nucleic acid promoter comprising SEQ ID NO:5, a fragment of SEQ ID NO:5, or a variant of SEQ ID NO: 5 having at least about 75% identity to SEQ ID NO: 5, that retains promoter activity in retinal cells. This promoter is designated the PACAP (pituitary adenylate cyclase activating polypeptide) promoter.

Table 1 provides the nucleotide sequences of the promoter-DREADD constructs and the PACAP promoter.

Table 1

SEQ ID NO:	Construct Name	Nucleotide Sequence
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SEQ ID NO:	Construct Name	Nucleotide Sequence
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SEQ ID NO:	Construct Name	Nucleotide Sequence
		gcaagcgaaa ggagaggagg cggctaatta aatattgagc agaaagtcgc gtggggagaa tgtcacgtgg gtctggaggc tcaaggaggc tgggataaat acgcgcaaggc actgagcagg cgaaagagcg cgctcggacc tccttcccgg cggcagctac cgagagtgcg gagcgaccag cgtgcgctcg gaggaaccag agaaactcag caccccgcgg gactgtccgt cgcagtaagt gggtaccgtc gacgccacca tgaccttgca caataacagt acaacctcgc ctttgtttcc aaacatcagc tcctcctgga tacacagccc ctccgatgca gggctgcccc cgggaaccgt cactcatttc ggcagctaca atgtttctcg agcagctggc aatttctcct ctccagacgg taccaccgat gaccctctgg gaggtcatac cgtctggcaa gtggtcttca tcgctttctt aacgggcatc ctggccttgg tgaccatcat cggcaacatc ctggtaattg tgtcatttaa ggtcaacaag cagctgaaga cggtcaacaa ctacttcctc ttaagcctgg cctgtgccga tctgattatc ggggtcattt caatgaatct gtttacgacc tacatcatca tgaatcgatg ggccttaggg aacttggcct gtgacctctg gcttgccatt gactgcgtag ccagcaatgc ctctgttatg aatcttctgg tcatcagctt tgacagatac ttttccatca cgaggccgct cacgtaccga gccaaacgaa caacaaagag agccggtgtg atgatcggtc tggcttgggt catctccttt gtcctttggg ctcctgccat cttgttctgg caatactttg ttggaaagag aactgtgcct ccgggagagt gcttcattca gttcctcagt gagcccacca ttacttttgg cacagccatc gctggttttt atatgcctgt caccattatg actattttat actggaggat ctataaggaa actgaaaagc gtaccaaaga gcttgctggc ctgcaagcct ctgggacaga ggcagagaca gaaaactttg tccaccccac gggcagttct cgaagctgca gcagttacga acttcaacag caaagcatga aacgctccaa caggaggaag tatggccgct gccacttctg gttcacaacc aagagctgga aacccagctc cgagcagatg gaccaagacc acagcagcag tgacagttgg aacaacaatg atgctgctgc ctccctggag aactccgcct cctccgacga ggaggacatt ggctccgaga cgagagccat ctactccatc gtgctcaagc ttccgggtca cagcaccatc ctcaactcca ccaagttacc ctcatcggac aacctgcagg tgcctgagga ggagctgggg atggtggact tggagaggaa agccgacaag ctgcaggccc agaagagcgt ggacgatgga ggcagttttc caaaaagctt ctccaagctt cccatccagc tagagtcagc cgtggacaca gctaagactt ctgacgtcaa ctcctcagtg ggtaagagca cggccactct acctctgtcc ttcaaggaag ccactctggc caagaggttt gctctgaaga ccagaagtca gatcactaag cggaaaagga tgtccctggt caaggagaag aaagcggccc agaccctcag tgcgatcttg cttgccttca tcatcacttg gaccccatac aacatcatgg ttctggtgaa caccttttgt gacagctgca tacccaaaac cttttggaat ctgggctact ggctgtgcta catcaacagc accgtgaacc ccgtgtgcta tgctctgtgc aacaaaacat tcagaaccac tttcaagatg ctgctgctgt gccagtgtga caaaaaaaag aggcgcaagc agcagtacca gcagagacag tcggtcattt ttcacaagcg cgcacccgag caggccttga aggatccacc ggtcgccacc atggtgagca agggcgagga ggataacatg gccatcatca aggagttcat gcgcttcaag gtgcacatgg agggctccgt gaacggccac gagttcgaga tcgagggcga gggcgagggc cgcccctacg agggcaccca gaccgccaag ctgaaggtga ccaagggtgg ccccctgccc ttcgcctggg acatcctgtc ccctcagttc atgtacggct ccaaggccta cgtgaagcac cccgccgaca tccccgacta cttgaagctg tccttccccg agggcttcaa gtgggagcgc gtgatgaact tcgaggacgg cggcgtggtg accgtgaccc aggactcctc cctgcaggac ggcgagttca tctacaaggt gaagctgcgc ggcaccaact tccctccga cggccccgta atgcagaaga agaccatggg ctgggaggcc tcctccgagc ggatgtaccc cgaggacggc gccctgaagg gcgagatcaa gcagaggctg aagctgaagg acggcggcca ctacgacgct gaggtcaaga ccacctacaa ggccaagaag cccgtgcagc tgcccggcgc ctacaacgtc aacatcaagt tggacatcac ctcccacaac gaggactaca ccatcgtgga acagtacgaa cgcgccgagg gccgccactc caccggcggc atggacgagc tgtacaagta agaattcgat atccagcaca gtggcggccg ctcgagtcta gagggccctt cgaaggtaag cctatcccta accctctcct cggtctcgat tctacgcgta ccggttaatc gataatcaac ctctggatta caaaatttgt gaaagattga ctggtattct taactatgtt gctccttta cgctatgtgg atacgctgct ttaatgcctt tgtatcatgc tattgcttcc cgtatggctt tcattttctc ctccttgtat aaatcctggt tgctgtctct ttatgaggag ttgtggcccg ttgtcaggca acgtggcgtg gtgtgcactg tgtttgctga cgcaaccccc actggttggg gcattgccac cacctgtcag ctcctttccg ggactttcgc tttccccctc cctattgcca cggcggaact catcgccgcc tgccttgccc gctgctggac aggggctcgg ctgttgggca ctgacaattc cgtggtgttg tcggggaaat catcgtcctt tccttggctg ctcgcctgtg

SEQ ID NO:	Construct Name	Nucleotide Sequence
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SEQ ID NO:11	PACAP-hM4D(Gi)-mCherry Construct	caatcttaaa ttttcaatta ttgcagaaaa cacagtgaca tggtttcaat ttttaaaact agtaagagcc acggagagtg tgaaagtgtg tagacaggaa aggtaaagat ccatctgaat actaggacta actcagaaga aaaagctttg cactgaggca gggattaagc aggttctgag cactgggaca ttcgtggaca cagaatccaa gggaagatta atatgaacag cggggtgatt tagacaatga actcccacag taagagcacc actgccaaag cttcaaattt agaggctgtg gtgaaaatta aaccagtggc aaatttcaac atttgcagca tctgcgccca aatagttcag caccaagagc ctggacagca ccacaggctc tcatcctagt ctcatccatc aatctattca gagacagact gtcaacccag ccagactcat tagatattta ctgaaaatcc ttataattct ttcctttaaa acacaaaacg acttccatgt ttagtagcct atttgaaaaa gcatatgcaa ggaattgaga gatcaaaatt aaaattatta ataggagatc ttgatggtgc ttaaatctag agatcagagt tgtcattggt gggggttgag tgaaaattaa gaaaaattag ggactcaata aaaacatgac ttcaccattc tctaaattct acgagttctt tacttgtctt tgagaaatca gtgaaatcga aaaccatcaa aataattgga cttcttaaaa attggattgt gtgagtgaaa ggtgtttatc agaagcggat gactccggat cttatcatcc tggaggactg cacagaatag ttaatatgtt ccttgaggga ctaggatgct gacgtctttt actgataccg gatcattacg tgactggggg agaaaaaaaa ggaagtcata tcatgaataa aaatcggagt gcaacagtgc aaccaaaata ttctgtactt gaaggcagaa agatgttgac aaagaggtgt ctcctgaaac cacgttcgga cagcttattt tgttaactgc atatataaaa acgagcagaa ggccagtgtc gacgccacca tggccaactt cacacctgtc aatggcagct cgggcaatca gtccgtgcgc ctggtcacgt catcatccca caatcgctat gagacggtgg aaatggtctt cattgccaca gtgacaggct ccctgagcct ggtgactgtc gtgggcaaca tcctggtgat gctgtccatc aaggtcaaca ggcagctgca gacagtcaac aactacttcc tcttcagcct ggcgtgtgct gatctcatca taggcgcctt ctccatgaac ctctacaccg tgtacatcat caagggctac tggccccctgg gcgccgtggt ctgcgacctg tggctggccc tggactgcgt ggtgagcaac gcctccgtca tgaaccttct catcatcagc tttgaccgct acttctgcgt caccaagcct ctcacctacc ctgcccggcg caccaccaag atggcaggcc tcatgattgc tgctgcctgg gtactgtcct tcgtgctctg ggcgcctgcc atcttgttct ggcagtttgt ggtgggtaag cggacggtgc ccgacaacca gtgcttcatc cagttcctgt ccaacccagc agtgaccttt ggcacagcca ttgctggctt ctacctgcct gtggtcatca tgacggtgct gtacatccac atctccctgg ccagtcgcag ccgagtccac aagcaccggc ccgagggccc gaaggagaag aaagccaaga cgctggcctt cctcaagagc ccactaatga agcagagcgt caagaagccc ccgcccgggg aggccgcccg ggaggagctg cgcaatggca agctggagga ggcccccccg ccagcgctgc caccgccacc gcgccccgtg gctgataagg acacttccaa tgagtccagc tcaggcagtg ccacccagaa caccaaggaa cgcccagcca cagagctgtc caccacagag gccaccacgc ccgccatgcc cgcccctccc ctgcagccgc gggccctcaa cccagcctcc agatggtcca agatccagat tgtgacgaag cagacaggca atgagtgtgt gacagccatt gagattgtgc ctgccacgcc ggctggcatg cgccctgcgg ccaacgtggc ccgcaagttc gccagcatcg ctcgcaacca ggtgcgcaag aagcggcaga tggcggcccg ggagcgcaaa gtgacacgaa cgatctttgc cattctgctg gccttcatcc tcacctggac gccctacaac gtcatggtcc tggtgaacac cttctgccag agctgcatcc ctgacacggt gtggtccatt ggctactggc tctgctacgt caacagcacc atcaaccctg cctgctatgc tctgtgcaac gccaccttta aaaagacctt ccggcacctg ctgctgtgcc agtatcggaa catcggcact gccaggcggg gatccccccgg tcgccaccat ggtgagcaag ggcgaggagg ataacatggc catcatcaag gagttcatgc gcttcaaggt gcacatggag ggctccgtga acggccacga gttcgagatc gagggcgagg gcgagggccg cccctacgag ggcacccaga ccgccaagct gaaggtgacc aagggtggcc cctgcccttc cgcctgggac atcctgtccc ctcagttcat gtacggctcc aaggcctacg tgaagcaccc cgccgacatc cccgactact tgaagctgtc cttccccgag ggcttcaagt gggagcgcgt gatgaacttc gaggacggcg gcgtggtgac cgtgacccag gactcctccc tgcaggacgg cgagttcatc tacaaggtga agctgcgcgg caccaacttc cctccgacg gccccgtaat gcagaagaag accatgggct gggaggcctc ctccgagcgg atgtaccccg aggacggcgc cctgaagggc gagatcaagc agaggctgaa

SEQ ID NO:	Construct Name	Nucleotide Sequence
		gctgaaggac ggcggccact acgacgctga ggtcaagacc acctacaagg ccaagaagcc cgtgcagctg cccggcgcct acaacgtcaa catcaagttg gacatcacct cccacaacga ggactacacc atcgtggaac agtacgaacg cgccgagggc cgccactcca ccggcggcat ggacgagctg tacaagtaag aattcgatat caagcttatc gataatcaac ctctggatta caaaatttgt gaaagattga ctggtattct taactatgtt gctccttta cgctatgtgg atacgctgct ttaatgcctt tgtatcatgc tattgcttcc cgtatggctt tcattttctc ctccttgtat aaatcctggt tgctgtctct ttatgaggag ttgtggcccg ttgtcaggca acgtggcgtg gtgtgcactg tgtttgctga cgcaacccc actggttggg gcattgccac cacctgtcag ctcctttccg ggactttcgc tttccccctc cctattgcca cggcggaact catcgccgcc tgccttgccc gctgctggac aggggctcgg ctgttgggca ctgacaattc cgtggtgttg tcggggaaat catcgtcctt tccttggctg ctcgcctatg ttgccacctg gattctgcgc gggacgtcct tctgctacgt ccctccggcc ctcaatccag cggaccttcc ttcccgcggc ctgctgccgg ctctgcggcc tcttccgcgt cttcgccttc gccctcagac gagtcggatc tccctttggg ccgcctcccc gcatcgatac cgagcgctgc tcgagagatc tacgggtggc atccctgtga cccctccca gtgcctctcc tggccctgga agttgccact ccagtgccca ccagccttgt cctaataaaa ttaagttgca tcattttgtc tgactaggtg tccttctata atattatggg gtggaggggg gtggtatgga gcaaggggca agttgggaag acaacctgta gggcctgcgg ggtctattgg gaaccaagct ggagtgcagt ggcacaatct tggctcactg caatctccgc ctcctgggtt caagcgattc tcctgcctca gcctcccgag ttgttgggat tccaggcatg catgaccagg ctcagctaat ttttgttttt ttggtagaga cggggtttca ccatattggc caggctggtc tccaactcct aatctcaggt gatctaccca ccttggcctc ccaaattgct gggattacag gcgtgaacca ctgctcccct ccctgtcct

Preferably, the nucleic acids hybridize under low, moderate or high stringency conditions. A first nucleic acid molecule is "hybridizable" to a second nucleic acid molecule when a single stranded form of the first nucleic acid molecule can anneal to the second nucleic acid molecule under the appropriate conditions of temperature and solution ionic strength (see Sambrook, *et al.*, supra). The conditions of temperature and ionic strength determine the "stringency" of the hybridization. Typical low stringency hybridization conditions include 55°C, 5X SSC, 0.1% SDS and no formamide; or 30% formamide, 5X SSC, 0.5% SDS at 42°C. Typical moderate stringency hybridization conditions are 40% formamide, with 5X or 6X SSC and 0.1% SDS at 42°C. High stringency hybridization conditions are 50% formamide, 5X or 6X SSC at 42°C or, optionally, at a higher temperature (e.g., 57°C, 59°C, 60°C, 62°C, 63°C, 65°C or 68°C). In general, SSC is 0.15M NaC1 and 0.015M Na-citrate. Hybridization requires that the two nucleic acids contain complementary sequences, although, depending on the stringency of the hybridization, mismatches between bases are possible. The appropriate stringency for hybridizing nucleic acids depends on the length of the nucleic acids and the degree of complementation, variables well known in the art. The greater the degree of similarity or homology between two nucleotide sequences, the higher the stringency under which the nucleic acids may hybridize. For hybrids of greater than 100 nucleotides in length, equations for

calculating the melting temperature have been derived (see Sambrook, *et al.*, supra, 9.50-9.51). For hybridization with shorter nucleic acids, *e.g.*, oligonucleotides, the position of mismatches becomes more important, and the length of the oligonucleotide determines its specificity (see Sambrook, *et al.*, supra, 11.7-11.8).

Designer Receptors Exclusively Activated by Designer Drugs (DREADDs)

Methods and compositions for treating diseases and disorders of the nervous system are provided. The methods employ the use of the chemogenetic tools known as Designer Receptors Exclusively Activated by Designer Drugs (DREADDs), to selectively control brain pathways that are initiated by the retina. Neurosurgical problems associated with injecting DREADDs into the brain can be avoided by using the retina as a target for DREADD expression.

The suprachiasmatic nucleus (SCN) receives non-image forming visual signals from the retina. These signals are then transmitted to the dorsal medial hypothalamus (DMH), which relays circadian information to the locus coeruleus (LC), together forming a circuit for the circadian regulation of arousal. Disruption to this circuit leads to degeneration of LC neurons and cortical noradrenergic-LC fibers, which leads to mood disturbance and other neurological disorders, including but not limited to cognitive deficits, loss of consciousness, and sleep and circadian disorders.

The above pathway may be referred to as the Photic Regulation of Arousal and Mood (PRAM) pathway. Disrupting this pathway leads to depressive-like behavior in rats. Depression is associated with altered circadian activity, e.g., blunted amplitude and phase delay of circadian rhythms, increased core temperature, and phase advanced oscillations of noradrenaline and cortisol plasma concentrations. Depression is also associated with disrupted sleep as well as dysregulation of sleep, e.g., seasonal affective disorder and short-day length lighting schedules.

Retinal expression of DREADDs has been demonstrated following intravitreal injection (IVI). The expression of DREADDs has been shown in retinal ganglion cells (RGCs) and in fibers of RGCs in suprachiasmatic nucleus (SCN), the region of the brain which controls circadian rhythms and which also affects mood, attention and cognitive processing. The function of DREADD expression in the retina has also been confirmed using electroretinogram (ERG) following delivery of a DREADD agonist, clozapine N-oxide (CNO). CNO efficacy was compared when administered systemically versus by eye drops, which represents a more

clinically useful administration technique. Significantly, application of CNO via eye drops is at least as successful at eliciting DREADD function as application of CNO via systemic delivery.

The present invention encompasses compositions and methods of inhibiting, activating, treating and/or preventing diseases and disorders of the nervous system in a subject, which involve the transfection of a class of receptors known as DREADDs. DREADDs are engineered G-protein coupled receptors which can be activated by otherwise inert drug-like small molecules. The technique has combined chemical and genetic approaches to achieve localized and temporally-specified decreases or increases (e.g., hM3Dq or hM4Di) in neuronal excitability by viral expression of the a particular DREADD. (Katzel et al. (2014) *Nat. Commun.*, 5:3847; Mahler et al. (2014) *Nat. Neurosci.*, 17:577-85; Pei et al. (2008) *Physiology* 23:313-21; Ferguson et al. (2011) *Nat. Neurosci.*, 14: 22–24; Fortress, A. M. et al. (2015) *J. Neurosci.*, *35*(4), 1343–1353; Vazey, E. M., et al. (2014). *Proc. Natl. Acad. Sci.*, *111*(10), 3859–3864; each of the foregoing references are incorporated by reference). In a particular embodiment, the methods comprise administering a nucleic acid molecule encoding a DREADD and administering an agonist of the DREADD to the subject.

For enhancing neuronal firing and activating Gq signaling in neuronal and non-neuronal cells, the hM3Dq DREADD is typically used (Alexander et al. (2009) *Neuron* 63(1):27-39.; Armbruster (2007) *Proc. Natl. Acad. Sci.,* 104(12):5163-8). The hM3Dq can be activated by clozapine-N-oxide (CNO), a pharmacologically inert metabolite of the atypical antipsychotic drug clozapine (Armbruster (2007) *Proc. Natl. Acad. Sci.,* 104(12):5163-8.); Roth et al (1994) *J. Pharmacol. Exp. Ther.* 268, 1403–1410.

The hM4Di receptor is a modified human muscarinic receptor that normally couples to Gi signaling cascades and GIRK channels, is insensitive to acetylcholine or other endogenous compounds (Pei et al. (2008) *Physiology* 23:313-21). However, this modified human muscarinic receptor is strongly activated by the otherwise pharmacologically inert ligand, clozapine N-oxide (CNO). Furthermore, specificity achieved by regional and cell-type specific expression of DREADDs which allows for targeted and temporally limited suppression of neuronal excitability.

The present invention uses the eye as a portal to influence brain activity and function, in particular to treat diseases and disorders of the nervous system, such as, for example, depression. Manipulation of the brain using DREADD injections into the eye to manipulate activity of

noradrenergic locus coeruleus neurons and to control the PRAM pathway and its associated pathways was neither suggested nor taught in the art. Likewise, the use of a PACAP promoter to drive DREADD expression in a specific subset of retinal ganglion cells was not taught or suggested in the art. The use of retinal stimulation of the PRAM pathway to manipulate and regulate activity of locus coeruleus neurons via natural circuit inputs allows for more physiological and clinically acceptable locus coeruleus activity patterns than would occur using prior methods of direct stimulaton by expression of DREADDs directly in locus coeruleus neurons. The present invention additionally provides the use of eye drops as a method to stimulate DREADD receptors that are expressed in the eye, a technique not taught or suggested in the art.

Thus, the present invention provides a genetically encoded and highly selective 'lock-and-key' approach to controlling aberrant neural function for therapeutic goals. Activation of DREADDs through the systemic or local delivery of an agonist, will attenuate neural hyperactivity, as well as adenylyl cyclase and cAMP levels, in neurons. DREADDs can be activated in a dose-dependent manner by their agonist, thereby allowing for flexible modulation of neuronal function.

There are several techniques to determine when retinal expression of the DREADD has occurred before administering the DREADD agonist to the subject. In one technique, a physician may use a SPECTRALIS® OCT platform and proceed with a technique called Optical Coherence Tomography (OCT) to detect expression of a DREADD. The expression of a DREADD can be successfully detected if it is tagged with a fluorescent marker, e.g., GFP, tdTomato, or mCherry. As a result, DREADD expression can be monitored prior to the administration of a DREADD agonist. In a second technique, agonist-dependent activation of a DREADD can be analyzed using an electroretinogram. In a third technique, positron emission tomography (PET) can be used to detect DREADD expression after an intravenous dose of a radiolabeled agonist such as an [^{11}C] agonist.

In one embodiment, a DREADD agonist may be administered immediately after administering a viral vector to the eye of a subject, wherein the viral vector comprises a promoter, a DREADD, and a 3' untranslated region encoded by the nucleotide sequence SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, or SEQ ID NO:11.

In another embodiment, the DREADD agonist may be administered one day after administering a viral vector to the eye of a subject, wherein the viral vector comprises a promoter, a DREADD, and a 3' untranslated region encoded by the nucleotide sequence SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, or SEQ ID NO:11.

In yet another embodiment, the DREADD agonist may be administered at three weeks, preferably four weeks, after administering a viral vector to the eye of a subject, wherein the viral vector comprises a promoter, a DREADD, and a 3' untranslated region encoded by the nucleotide sequence SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, or SEQ ID NO:11.

In another embodiment, the DREADD agonist may be administered prophylactically, prior to, at the same time, or a time after administering a viral vector to the eye of a subject, wherein the viral vector comprises a promoter, a DREADD, and a 3' untranslated region encoded by the nucleotide sequence SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, or SEQ ID NO:11.

In another embodiment, the described viral vector comprising a promoter, a DREADD, and a 3' untranslated region encoded by the nucleotide sequence SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, or SEQ ID NO:11, may be co-administered with an anti-inflammatory agent, which may be selected from a systemically-injected corticosteroid, an intravitreally-injected ketorolac, an intravitreally-injected diclofenac or other clinically acceptable anti-inflammatory agent.

The methods of the present invention may also comprise administering at least one other therapeutic agent for the treatment of a nervous system disease or disorder, which include acamprosate, agomelatine, alimemazine, alprazolam, amantadine, amfetamine, amisulpride, amitriptyline, amobarbital, amoxapine, apomorphine, apomorphine, aripiprazole, asenapine, atomoxetine, atropine, baclofen, benperidol, benztropine, biperiden, bromazepam, bromocriptine, bromperidol, brotizolam, buprenorphine, bupropion, buspirone, butobarbital, cabergoline, carbamazepine, chloral hydrate, chlordiazepoxide, chlorpheniramine, chlorpromazine, chlorprothixene, citalopram, clobazam, clomethiazole, clomipramine, clonazepam, clonidine, clorazepate, clozapine, cyclobarbital, cyproheptadine, cytisine,

desipramine, desvenlafaxine, dexamfetamine, dexmethylphenidate, dextromethorphan, diazepam, dicyclomine dimenhydrinate, diphenhydramine, disulfiram, divalproex sodium, donepezil, doxacurium, doxepin, doxylamine, duloxetine, edaravone, electroconvulsive therapy, enanthate, escitalopram, estazolam, eszopiclone, ethosuximide, flunitrazepam, fluoxetine, flupenthixol, fluphenazine, flurazepam, fluspirilen, fluvoxamine, gabapentin, galantamine, glutethimide, glycopyrrolate, guanfacine, haloperidol, hexamethonium, hydrochloride, hydroxyzine, iloperidone, imipramine, ipratropium, lamotrigine, levetiracetam, levodopa, levomepromazine, levomilnacipran, lisdexamfetamine, lisuride, lithium salts, loprazolam, lorazepam, lormetazepam, mecamylamine, melatonin, melperone, memantine, meprobamate, metamfetamine, methadone, methylphenidate, mianserin, midazolam, mirtazapine, moclobemide, modafinil, modecate, motherwort, nalmefene, naltrexone, niaprazine, nimetazepam, nitrazepam, nortriptyline, olanzapine, omca, ondansetron, orphenadrine, oxazepam, oxcarbazepine, oxitropium, oxybutynin, paliperidone, paroxetine, penfluridol, pentobarbital, perazine, pergolide, pericyazine, perphenazine, phenazepam, phenelzine phenobarbital, phenytoin, pimozide, piribedil, pramipexole, pregabalin, prolixin decanoate, promethazine, propantheline bromide, prothipendyl, protriptyline, quazepam, quetiapine, ramelteon, rasagiline, reboxetine, remacemide, reserpine, riluzole, risperidone, rivastigmine, ropinirole, rotigotine, rubidium chloride, safinamide, scopolamine, secobarbital, sediten, selecten, selegiline, selegiline, sertindole, sertraline, sertraline, sevinol, sinqualone enantat, siqualone, sirtal, sodium oxybate, sodium valproate, solifenacin, stazepine, stelazine, sulpiride, suvorexant, tacrine, tegretol, telesmin, temazepam, terfluzine, tetrabenazine, thioridazine, thiothixene, tianeptine, timonil, tiotropium, tizanidine, tofisopam, tolcapone, tolterodine, topiramate, trancin, tranylcypromine, trazodone, triazolam, trifluoperaz, trifluoperazine, triftazin, trihexyphenidyl, trimipramine, tropicamide, tubocurarine, valerian, valproate, valproic acid, varenicline, venlafaxine, vilazodone, vortioxetine, zaleplon, ziprasidone, zolpidem, zopiclone, zotepine, zuclopenthixol or other neuroeptic, antidepressant or pharmacotherapeutic agents for treating any of the aforementioned disorders.

In a particular embodiment, the DREADD nucleic acid and/or DREADD agonist is delivered as a composition with at least one pharmaceutically acceptable carrier.

In some embodiments, the disease or disorder of the nervous system is a neuropsychiatric disorder. Individuals may be identified as having a neuropsychiatric disorder using the criteria

set forth in the American Psychiatric Association's Diagnostic and Statistical Manual of Mental Health (DSM-5). The DSM-5 identifies disorders which are classified as neuropsychiatric disorders. Examples of neuropsychiatric disorders include: depression, seasonal affective disorder, anxiety, sleep and circadian disorders, stress disorders including Post Traumatic Stress Disorder (PTSD), Attention Deficit Hyperactivity Disorder (ADHD), autism, addiction, epilepsy, and Intensive Care Unit (ICU) psychosis.

In another embodiment, the sleep disorder is desynchronosis ("jet lag"), which is a temporary disorder that causes fatigue, insomnia, and other symptoms because of air travel across time zones. It is considered a circadian rhythm sleep disorder, which is a disruption of the internal body clock.

In some embodiments, the disease or disorder of the nervous system is a neurodegenerative disease, which includes amyotrophic lateral sclerosis (ALS), Parkinson's disease, Alzheimer's disease, and Huntington's disease.

In another embodiment, the disease or disorder of the nervous system is a cerebrovascular accident (CVA) or stroke. A DREADD nucleic acid and/or DREADD agonist may be administered to a subject to alleviate the symptoms of a cerebrovascular accident (CVA) or stroke. These symptoms include the subject having trouble remaining conscious, walking, speaking, and understanding, as well as paralysis or numbness of the face, arm, or leg.

In another embodiment, a DREADD nucleic acid and/or DREADD agonist may be administered to a subject, while the subject is treated with an additional therapeutic method for a disease or disorder of the nervous system. These additional therapeutic methods include, but are not limited to, cognitive behavioral therapy, light therapy, and electroconvulsive therapy. For example, a subject may receive a DREADD agonist while receiving light therapy for a neuropsychiatric disorder. The combined treatment methods may result in augmenting the effectiveness of the light therapy and / or reduce the frequency and dosages of light therapy protocols.

Table 2 describes diseases and disorders of the nervous system, the brain region affected, treatments, and predicted outcomes.

Table 2

Disease/Disorder of the Nervous System	Brain Region Affected	Treatment	Predicted Outcome
Depression	Dysregulated SCN, Dysregulated DMH, hypoactive LC (Bowrey et al. *Depress. Anxiety.* 2017)	Activate retina with AAV2-hSyn-hM3Dq or AAV2-PACAP-hM3Dq.	Construct will restore (increase) normal firing of LC and reverse depression symptoms.
Seasonal Affective Disorder	Dysregulated SCN, Dysregulated DMH, hypoactive LC (Bowrey et al. *Depress. Anxiety.* 2017)	Activate retina with AAV2-hSyn-hM3Dq or AAV2-PACAP-hM3Dq.	Construct will restore (increase) normal firing of LC and reverse depression symptoms.
Stress Disorder	Hyperactive noradrenergic/LC system (Valentino RJ, Foote SL *J Neurosci.* 1988 Mar; 8(3):1016-25; Bremner et al. *Synapse.* 1996 pp 23: 39-51)	Inhibit retina with AAV2-hSyn-hM4Di or AAV2-PACAP-hM4Di.	Construct will restore (decrease) normal firing of LC and prevent stress symptoms.
Anxiety	Hyperactive noradrenergic/LC system (Bremner et al. *Synapse.* 1996 pp 23: 39-51)	Inhibit retina with AAV2-hSyn-hM4Di or AAV2-PACAP-hM4Di.	Construct will restore (decrease) normal firing of LC and prevent anxiety symptoms.
Autism	Dysregulation of LC firing (*Annu. Rev. Neurosci.* 2005.28:403-450)	Activate retina with AAV2-hSyn-hM3Dq or AAV2-PACAP-hM3Dq.	Construct will enhance tonic firing of LC, reducing autism-related deficits in behavioral flexibility.
Substance Use Disorder	Hyperactivity of LC during withdrawal from drug (Hooshmand et al. *Neuroscience Letters* 636, 276-281)	Inhibit retina with AAV2-hSyn-hM4Di or AAV2-PACAP-hM4Di.	Construct will restore (decrease) normal firing of LC and prevent withdrawal symptoms.
Epilepsy	Anti-epileptic treatments appear to be mediated by LC (Fornai et al. *EJN.* 33: 2169–2178, 2011)	Activate retina with AAV2-hSyn-hM3Dq or AAV2-PACAP-hM3Dq to activate LC.	Secondary LC activation will enhance antiepileptic treatments such as vagus nerve stimulation.
Intensive Care Unit Psychosis	Dysregulation of SCN (Barrosso et al. *Lighting Res. Technol.* 2013; 45: 197–216)	Activate retina with AAV2-hSyn-hM3Dq or AAV2-PACAP-hM3Dq to activate LC at dawn.	Retina activation at dawn will synchronize SCN, improving psychosis symptoms.
Desynchronosis ("jet lag")	When travelling across time zones, the body clock is out of synchronization with the destination time	Activate retina with AAV2-hSyn-hM3Dq or AAV2-PACAP-hM3Dq at dawn of the	Retina activation at dawn of the destination time will synchronize SCN, improving jetlag symptoms.

Disease/Disorder of the Nervous System	Brain Region Affected	Treatment	Predicted Outcome
		destination time.	
Amyotrophic Lateral Sclerosis	Bunina bodies in the locus ceruleus pigmented neurons (Iwanaga et al. *Clin Neuropathol.* 1997; 16:23-6)	Activate retina with AAV2-hSyn-hM3Dq or AAV2-PACAP-hM3Dq at dawn of the destination time.	Secondary LC activation will reduce ALS symptoms.
Parkinson's Disease	Lewy pathologyand cell loss in the LC precedes that of other key PD-relevant brain structures (Vermeiren. Neurochemistry International Volume 102, January 2017, Pages 22-32)	Chronically activate retina with AAV2-hSyn-hM3Dq or AAV2-PACAP-hM3Dq.	Retinal activation will be protective against the development of lewy pathology in the LC and will activate remaining LC neurons to compensate for lost neurons.
Alzheimer's Disease	Loss of locus coeruleus neurons (Bondareff et al. *Lancet*. 1981 Apr 4; 1(8223):783-4)	Chronically activate retina with AAV2-hSyn-hM3Dq or AAV2-PACAP-hM3Dq.	Retinal activation will reduce loss of locus coeruleus neurons, and activate remaining neurons to compensate for lost neurons, reducing cognitive symptoms of Alzheimer's disease.
Huntington's Disease	Reduced cell numbers and length of locus coeruleus (Zweig et al. *Archives of Neurology*. 1992. 49(2):152-6)	Chronically activate retina with AAV2-hSyn-hM3Dq or AAV2-PACAP-hM3Dq.	Retinal activation will reduce loss of locus coeruleus neurons, and activate remaining neurons to compensate for lost neurons, slowing the time course of symptoms of Huntington's disease.
Stroke	Reductions in norepinephrine in LC following cerebral infarction (Robinson R.G., 1979. *Science*. 205: pp. 707-710)	Chronically activate retina with AAV2-hSyn-hM3Dq or AAV2-PACAP-hM3Dq.	Secondary LC activation will increase norepinephrine, improving symptom outcomes.

The nucleic acids of the present invention may be delivered to a cell, e.g., neuron, by any known method. For example, the nucleic acids can be delivered via synthetic delivery systems, liposomes, nanoparticles, or viral vectors. The nucleic acid molecules encoding the DREADD may be contained within an expression vector, particularly a viral vector such as an adeno-associated viral vector. The nucleic acid molecules (or vectors) may be directly delivered to the target site, e.g., by microinjection. For example, the nucleic acid molecules or vectors may be administered, e.g., by injection to the intraocular space and/or retina. Intraocular injections may

include intravitreal injections, sub-internal limiting membrane injections, and sub-retinal injections.

Examples of viral vectors include, without limitation, lentiviral, retroviral, herpesviral, e.g., replication-defective herpes simplex virus (HSV), adenoviral, and adeno-associated viral vectors. In a particular embodiment, the vector is an AAV vector. The AAV vector can be of any AAV serotype. For example, the AAV vector can be, without limitation, AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV11, AAV12, and hybrids thereof. For example, an AAV vector can be a combinatorial hybrid of 2, 3, 4, 5, or more serotypes. AAV vectors can have one or more of the AAV wild-type genes deleted in whole or part. In a particular embodiment, the AAV vector is AAV2.

As explained above, DREADDs are engineered G-protein coupled receptors which are activated by otherwise inert drug-like small molecules (reviewed in Urban et al. (2015) *Ann. Rev. Pharmacol.Toxicol.*, 55: 399-417; incorporated herein by reference). Methods of generating DREADDs are known in the art (see, e.g., Dong et al. (2010) *Nat. Protoc.*, 5(3):561-73).

In a particular embodiment, the DREADD is based on the muscarinic receptor (e.g., human muscarinic receptor). In another particular embodiment, the DREADD is a KORD (kappa opioid receptor-DREADD (e.g., human KOR). For example, the KORD may be a G-protein coupled (e.g., Gi-coupled) kappa-opioid receptor DREADD wherein the inert ligand or agonist is salvinorin B (salB). In a particular embodiment, the DREADD is coupled with Gi.

In a particular embodiment, the DREADD is hM4Di (human M4 muscarinic cholinergic Gi-coupled DREADD. In a particular embodiment, the DREADD is human muscarinic acetylcholine receptor M4 (e.g., GenBank Accession No. NP_000732, Gene ID: 1132) comprising two-point mutations: a substitution at Y113 (e.g., Y113C) and a substitution at A203 (e.g., A203G). PCT Publication No. WO 2015/136247 (incorporated herein by reference) also provides a nucleic acid sequence encoding hMD4i. A plasmid encoding hM4Di is available commercially as plasmid 45548 (Addgene, Cambridge, MA, www.addgene.org/45548).

In a particular embodiment, the DREADD is coupled with Gq. In a particular embodiment, the DREADD is Gq-coupled human M3 muscarinic receptor (hM3Dq) (see, e.g., Alexander et al. (2009) *Neuron* 63(1): 27–39; Armbruster et al. (2007) *Proc. Natl. Acad. Sci.*, 104(12):5163–5168; Alexander et al. (2009) *Neuron* 63(1):27–39). A plasmid encoding hM3Dq

is available commercially as plasmid 44361 (Addgene, Cambridge, MA, www.addgene.org/44361).

In a particular embodiment, the agonist is clozapine N-oxide, DREADD agonist 21 (Tocris Bioscience, Bristol, UK; 11-(1-piperazinyl)-5H-dibenzo[b,e][1,4]diazepine), salvinorin B, clozapine, olanzapine, or perlapine (Tocris Bioscience, Bristol, UK; 6-(4-methyl-1-piperazinyl)-11H-dibenz[b,e]azepine); Chen et al. (2015) *ACS Chem. Neurosci.*, 6(3):476-84).

The nucleic acids encoding DREADDs may be under the control of a neuron specific promoter. In a particular embodiment, the neuron-specific promoter is synapsin (e.g., Kugler et al. (2003) *Gene Ther.*, 10:337-47). The synapsin promoter (e.g., human synapsin promoter) drives production of a DREADD (e.g., hM4D(Gi) or hMD3(Dq)) in a large percentage of neurons.

In a particular embodiment, the neuron-specific promoter is a pituitary adenylate cyclase activating polypeptide (PACAP) promoter, a melanopsin promoter, or a promoter that expresses in retinal neurons that project to SCN.

In another embodiment, the neuron-specific promoter is PRSx8. PRSx8 is based on an upstream regulatory site in the human DBH promoter and drives high levels of expression in adrenergic neurons.

In yet another embodiment, the neuron-specific promoter is preprotachykinin-1 promoter (TAC-1).

The nucleic acid sequences of several promoter-driven DREADDs are described. They include, but are not limited to, PACAP-hM3D(Gq)-mCherry (SEQ ID: 1), TAC-1-hM4D(Gi)-mCherry (SEQ ID NO:2), PRSx8-HA-hM3D(Gq) (SEQ ID NO:3), PRSx8-HA-hM4D(Gi) (SEQ ID NO:4), PACAP-hM3D(Gq) (SEQ ID NO:6), PRSx8-hM3D(Gq) (SEQ ID NO:7), PRSx8-hM4D(Gi) (SEQ ID NO:8), and TAC-1-hM4D(Gi) (SEQ ID NO:9), TAC-1-hM3D(Gq)-mCherry (SEQ ID NO:10) and PACAP-hM4D(Gi)-mCherry (SEQ ID NO:11). The agonist of a DREADD preferentially binds and activates the administered DREADD receptor over other receptors (e.g., a selective agonist). It is desirable to use a DREADD agonist which is inert or has little or no biological effects other than stimulating the DREADD. For example, the agonist may be a ligand of the DREADD.

In a particular embodiment, the agonist is clozapine N-oxide (CNO) or salvinorin B (salB). The DREADD agonist may be delivered systemically to the subject (e.g., orally, topically (e.g., to the skin) or directly to the eye (e.g., injection or eye drops).

Pharmaceutical Compositions and Administration

The composition may be administered by any suitable means, including ocular, oral, parenteral, intramuscular, intravenous, intra-arterial, intraperitoneal, subcutaneous, topical, inhalatory, transdermal, intrapulmonary, intrarectal, intramuscular, and intranasal administration.

In one particular embodiment, the nucleic acid composition is administered by injection (e.g., microinjection to the retina, intravitreal injection, etc.).

An example of administering a DREADD to the eye of mice has been described (Li et al. (2016). *Proc. Natl. Acad. Sci.*, 113(7):1937–1942. In this report, Li et al. injected an AAV vector expressing a DREADD (a mutant M3 muscarinic G protein-coupled receptor (GPCR) hM3Dq to selectively activate Gq/11 signaling) into the eyes of mice using methods described in Park et al. (2008) *Science* 322(5903):963–966. The Gq/11-coupled designer GPCR in Li could not be activated by its natural ligand (acetylcholine), but became activated by clozapine N-oxide (CNO), an otherwise pharmacologically inert compound (Farrell et al. (2013) *Brain Res* 1511:6–20). Briefly, mice were anaesthetized with ketamine and xylazine and the vector was injected into the vitreous bodies using a glass micropipette coupled to a Hamilton microsyringe. The micropipette was inserted in the peripheral retina, just behind the ora serrata, and was deliberately angled to avoid damage to the lens.

In another particular embodiment, the composition comprising the agonist is administered systemically (e.g., orally), topically (e.g., to the skin) or to the eye (e.g., via eye drops).

Compositions comprising the DREADD agonist, e.g., clozapine N-oxide, DREADD agonist 21, salvinorin B, clozapine, olanzapine, or perlapine, and a carrier are encompassed by the present invention, particularly compositions suitable for ocular administration, particularly topical administration to the eye, for example, sterile eye drops or ointment. In a particular embodiment, the composition is an aqueous formulation with a pH physiologically compatible with the eye (e.g., a pH in the range from about 4 to about 8, about 5.5 to about 8, or about 6.0 to about 7.5).

In a particular embodiment, the composition is an aqueous formulation having isotonic and physiological characteristics suitable for ocular administration. The compositions may also be modified to increase the residence time of the compounds in the eye, provide a sustained release of compounds, and/or avoid toxicity and increase ocular tolerability.

In a particular embodiment, the tonicity of the composition approximates physiological tonicity (e.g., 0.9% saline). Compounds such as, without limitation: sodium chloride, potassium chloride, calcium chloride, dextrose and/or mannitol, may be added. In a particular embodiment, the osmolality of the composition is about 150 to about 450 mOsm or about 250 to about 350 mOsm.

In a particular embodiment, the composition may further comprise a compound which soothes the eye; reduces surface tension and improves wettability; and/or enhances the viscosity of the composition (e.g., to a viscosity of about 10 to about 100 or about 25 to about 50 centipolses), such agents include, without limitation: polyols (e.g., tyloxapol, glycerol, propylene glycol, ethylene glycol, polyethylene glycol), cellulose derivatives (e.g., hydroxyethylcellulose, hypromellose, hydroxypropylmethyl cellulose, methylcellulose, carboxymethylcellulose sodium, hydroxylpropylcellulose), dextran, gelatin, vinyl polymers (e.g., polyvinyl alcohol, polyvinyl pyrrolidine), polysorbate 80, povidone, carbomers, and polysaccharides/glycosaminoglycans (e.g., hyaluronan, chondroitin sulfate).

In a particular embodiment, the composition comprises an antioxidant. In a particular embodiment, the composition comprises a preservative (e.g., quaternary ammonium salts (e.g., benzalkonium chloride, benzethonium chloride, cetalkonium chloride, cetrimide, benzododecinium bromide and benzoxonium chloride), alkyl-mercury salts of thiosalicylic acid, parabens, chelating agents, chlorobutanol, boric acid, sorbic acid, phenylethanol, and the like).

In general, the pharmaceutically acceptable carrier of the composition is selected from the group of diluents, preservatives, solubilizers, emulsifiers, adjuvants and/or carriers. The compositions can include diluents of various buffer content (e.g., Tris HCl, acetate, phosphate), pH and ionic strength; and additives such as detergents and solubilizing agents (e.g., polysorbate 80), anti-oxidants (e.g., ascorbic acid, sodium metabisulfite), preservatives (e.g., Thimerosal, benzyl alcohol) and bulking substances (e.g., lactose, mannitol). The compositions can also be incorporated into particulate preparations of polymeric compounds such as polyesters, polyamino acids, hydrogels, polylactide/glycolide copolymers, ethylenevinylacetate copolymers,

polylactic acid, polyglycolic acid, etc., or into liposomes. Such compositions may influence the physical state, stability, rate of in vivo release, and rate of in vivo clearance of components of a pharmaceutical composition of the present invention (see, e.g., Remington's Pharmaceutical Sciences and Remington: The Science and Practice of Pharmacy). The pharmaceutical composition of the present invention can be prepared, for example, in liquid form, or can be in dried powder form (e.g., lyophilized for later reconstitution).

The therapeutic agents described will generally be administered to a patient as a pharmaceutical preparation. The term "patient" refers to human or animal subjects. The compositions of the present invention may be employed therapeutically or prophylactically, under the guidance of a physician or veterinarian.

The compositions comprising the agent of the present invention may be conveniently formulated for administration with any pharmaceutically acceptable carrier(s). The concentration of agent in the chosen medium may be varied and the medium may be chosen based on the desired route of administration of the pharmaceutical preparation. Except insofar as any conventional media or agent is incompatible with the agent to be administered, its use in the pharmaceutical preparation is contemplated.

The dose and dosage regimen of the agent according to the present invention that is suitable for administration to a particular patient may be determined by a physician considering the patient's age, sex, weight, general medical condition, and the specific condition for which the agent is being administered to be treated or prevented and the severity thereof. The physician may also take into account the route of administration, the pharmaceutical carrier, and the agent's biological activity. Selection of a suitable pharmaceutical preparation will also depend upon the mode of administration chosen.

A pharmaceutical preparation of the present invention may be formulated in dosage unit form for uniformity and for ease of administration. The dosage unit form refers to the physical discrete unit of the pharmaceutical preparation appropriate for a patient undergoing treatment therapy. Each dosage should contain a quantity of active ingredient, e.g., DREADD agonist, calculated to produce the desired effect in association with a selected pharmaceutical carrier.

Procedures for determining the appropriate dosage unit are well known to those skilled in the art. For example, dosage units may be proportionately increased or decreased based on the weight of the subject. Appropriate concentrations for alleviation or prevention of a particular

condition (disease or disorder of the nervous system) may be determined by dosage concentration curve calculations, as known in the art.

In one embodiment, the concentration of the therapeutic agent, e.g., DREADD agonist, will range from 0.1 mg / kg – 100 mg / kg depending on subject species.

After administration of the DREADD, a pharmaceutical preparation comprising the therapeutic agent, e.g. DREADD agonist, may be administered at appropriate intervals until the pathological symptoms are reduced or alleviated, after which the dosage may be reduced to a maintenance level.

The first dose of the of a DREADD agonist may be administered immediately after intraocularly or intravitreally administering a viral vector, wherein the viral vector comprises a promoter, a DREADD, and a 3' untranslated region encoded by the nucleotide sequence SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, or SEQ ID NO:11.

In another embodiment, the DREADD agonist may be administered one day after intraocularly or intravitreally administering a viral vector, wherein the viral vector comprises a promoter, a DREADD, and a 3' untranslated region encoded by the nucleotide sequence SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, or SEQ ID NO:11.

In yet another embodiment, the DREADD agonist may be administered at three weeks, preferably four weeks, after intraocularly or intravitreally administering a viral vector, wherein the viral vector comprises a promoter, a DREADD, and a 3' untranslated region encoded by the nucleotide sequence SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, or SEQ ID NO:11.

The appropriate time to administer the DREADD agonist may be determined by three methods known in the art. The first method visualizes DREADD expression using optimal coherence tomography (OCT). The second method is through the analysis of an electroretinogram wave during agonist-dependent activation of a DREADD. The third method occurs by using positron emission tomography (PET) after an intravenous dose of a radiolabeled agonist such as an [^{11}C] agonist. It is expected that the optimal first dose of agonist may be within the range of 3 – 4 weeks following administration of the DREADD nucleic acid molecules.

In another embodiment, the pharmaceutical preparation comprising the therapeutic agent, e.g., DREADD agonist, may be administered at appropriate daily intervals, for example, at least once per day, at least twice per day, at least three times per day, or until pathological symptoms of a disease or disorder of the nervous system are reduced or alleviated.

The appropriate daily interval of administration of the therapeutic agent, e.g., DREADD agonist, may depend on the condition of the patient, e.g., severity of nervous system disease or disorder.

Toxicity and efficacy, for example, therapeutic, preventative, of the particular formulas described can be determined by standard pharmaceutical procedures such as *in vitro*, in cell cultures, *ex vivo*, or on experimental animals. The data obtained from these studies can be used in formulating a range of dosage for use in human. The dosage may vary depending upon form and route of administration. Dosage amount and interval may be adjusted individually to levels of the active ingredient which are sufficient to deliver a therapeutically or prophylactically effective amount.

Kits

The present invention also provides kits comprising one or more components including, but not limited to, the viral vectors, promoter, and DREADD, as discussed, in association with one or more additional components including, but not limited to, a pharmaceutically acceptable carrier and the DREAD agonist. The viral vectors, promoter, DREADD composition and/or the DREAD agonist can be formulated as pure compositions or in combination with a pharmaceutically acceptable carrier, in a pharmaceutical composition.

Kits may also include primers, buffers, and probes along with instructions for determining elevated levels of nucleic acid, proteins, or protein fragments of the DREADDs.

In one embodiment, a kit includes a viral vector, a promoter, a DREADD composition of the invention or a pharmaceutical composition thereof in one container and a DREADD agonist or a pharmaceutical composition thereof in another container (*e.g.*, in a sterile glass or plastic vial).

If the kit includes a pharmaceutical composition for parenteral administration to a subject, the kit can include a device for performing such administration. For example, the kit can include one or more hypodermic needles or other injection devices as discussed above.

The kit can include a package insert including information concerning the pharmaceutical compositions and dosage forms in the kit. Generally, such information aids patients and physicians in using the enclosed pharmaceutical compositions and dosage forms effectively and safely. For example, the following information regarding a combination of the invention may be supplied in the insert: pharmacokinetics, pharmacodynamics, clinical studies, efficacy parameters, indications and usage, contraindications, warnings, precautions, adverse reactions, overdosage, proper dosage and administration, how supplied, proper storage conditions, references, manufacturer/distributor information and patent information.

General Methods

Standard methods in molecular biology are described in Sambrook, Fritsch and Maniatis (1982 & 1989 2nd Edition, 2001 3rd Edition) *Molecular Cloning, A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY; Sambrook and Russell (2001) *Molecular Cloning, 3rd ed.,* Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY; Wu (1993) *Recombinant DNA*, Vol. 217, Academic Press, San Diego, CA). Standard methods also appear in Ausbel, *et al.* (2001) *Current Protocols in Molecular Biology, Vols.1-4*, John Wiley and Sons, Inc. New York, NY, which describes cloning in bacterial cells and DNA mutagenesis (Vol. 1), cloning in mammalian cells and yeast (Vol. 2), glycoconjugates and protein expression (Vol. 3), and bioinformatics (Vol. 4).

Methods for protein purification including immunoprecipitation, chromatography, electrophoresis, centrifugation, and crystallization are described (Coligan, *et al.* (2000) *Current Protocols in Protein Science, Vol. 1*, John Wiley and Sons, Inc., New York). Chemical analysis, chemical modification, post-translational modification, production of fusion proteins, glycosylation of proteins are described (see, *e.g.*, Coligan, *et al.* (2000) *Current Protocols in Protein Science, Vol. 2*, John Wiley and Sons, Inc., New York; Ausubel, *et al.* (2001) *Current Protocols in Molecular Biology, Vol. 3*, John Wiley and Sons, Inc., NY, NY, pp. 16.0.5-16.22.17; Sigma-Aldrich, Co. (2001) *Products for Life Science Research*, St. Louis, MO; pp. 45-89; Amersham Pharmacia Biotech (2001) *BioDirectory*, Piscataway, N.J., pp. 384-391). Production, purification, and fragmentation of polyclonal and monoclonal antibodies are described (Coligan, *et al.* (2001) *Current Protocols in Immunology, Vol. 1*, John Wiley and Sons, Inc., New York; Harlow and Lane (1999) *Using Antibodies*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY; Harlow and Lane, *supra*). Standard techniques for

characterizing ligand/receptor interactions are available (see, *e.g.*, Coligan, *et al.* (2001) *Current Protocols in Immunology, Vol. 4*, John Wiley, Inc., New York).

EXAMPLES

Example 1

The Excitatory hM3Di DREADD in the Retina

Weight loss is a common feature of depression in humans and depression-like behavior in animals (American Psychiatric Association, DSM-5, 2013; Liu et al. *Behavior. Brain Res.*, 305:148–156, 2016). The effect of DREADD activation on depression-associated weight loss was studied in rats.

Rats were administered AAV vectors with the human synapsin promoter (hSyn) encoding hM3Dq (G(q)) DREADD (AAV-hSyn-hM3Di) or control vector (hSyn-GFP) by intravitreal injection (IVI). After allowing 4 weeks for expression of the hM3Di DREADD, the rats were housed in total darkness for 24 hours a day. The total darkness exposure is an environmental condition which leads to depression-like behavior in animals (Gonzalez et al. *Proc. Natl. Acad. Sci.*, 105(12):4898-903, 2008). The agonist CNO was administered once per day to activate retinal cells in in animals expressing hM3Di DREADD only.

Figure 1 shows weight loss in all animals for the first three days of total dark-rearing. In the following four days, the animals with hM3D1 DREADD-activated retinas recovered from weight loss, while animals administered hSyn-GFP control vectors continued to lose weight. The recovery from weight loss indicated that hM3Di DREADD-activated retinas reduced depression-associated behavior induced by constant dark-rearing.

Example 2

DREADD Expression and Function in Retinal Cells via Intravitreal Injection

Figure 2 shows DREADD expression four weeks following DREADD administration. Rats were intravitreally administered AAV-hSyn-hM4Di. Figure 2A shows transfections of the DREADD in retinal cells. Figure 2B shows DREADD terminal transport to suprachiasmatic nucleus. Following confirmed DREADD expression, electoretinography (ERG) was performed. Figure 2C shows inhibition of the ERG wave 20 minutes following CNO agonist administration (by intraperitoneal injection), indicating decreased retinal activity. Figure 2D shows DREADD activation with eye drops. Eye drops were administered and ERG readings were recorded 10

minutes and 20 minutes thereafter. Similar to systemic delivery, eyedrops decreased function in retinal cells at both timepoints.

Example 3

DREADD Activation of Retinal Cells Prevents the Development of Light Deprivation-induced Depression-like Behavior

Rats were administered AAV vectors encoding hM3Dq (G(q)) with the hSyn promoter(AAV-hSyn-hM3Di) or control vector (h-Syn-GFP) by intravitreal injection (IVI) or no injection. After allowing 4 weeks for expression of the hM3Di DREADD, the rats were housed in total darkness for 24 hours a day. The total darkness exposure is an environmental condition which leads to depression-like behavior in animals (Gonzalez et al. *Proc. Natl. Acad. Sci.*, 105(12):4898-903, 2008). The agonist CNO was administered once per day to activate retinal cells in in animals expressing hM3Di DREADD only.

Figure 3 shows DREADD activation of retinal cells preventing the development of light deprivation-induced depression-like behavior. Figure 3A shows percentage of weight change in all animals for the first eight weeks of total dark-rearing. GFP animals lost weight, control animals failed to gain weight, and hM3Dq animals gained weight, as expected. The recovery from weight loss indicates that DREADD-activated retinas reduced depression-associated behavior induced by constant dark-rearing. Behavioral measures of other depression like behavior was measured. The saccharin preference test showed control and GFP animals preferred saccharin less than hM3Dq animals (Figure 3B) and hM3Dq animals had greater swimming and less immobility during the forced swim test (Figure 3C) that both control groups, indicating the absence of depression-like behavior in hM3Dq animals. The construct did not induce anxiety like behaviors (Figure 3D and Figure 3E), and there was no effect on general locomotor behavior (Figure 3F).

Example 4

DREADD Activation of Retinal Cells in Constant Darkness Prevents Apoptosis in Locus Coeruleus

Three groups of rats were either administered AAV vectors encoding hM3Dq (G(q)) with the hSyn promoter or control vector (GFP) by intravitreal injection (IVI) or no injection (DD-Control). After 4 weeks to allow for expression of the excitatory DREADD, the rats were housed in total darkness for 24 hours a day. The agonist CNO was administered once per day to

both hM3Dq and GFP animals, activating retinal cells in G(q) animals only. A fourth group of rats was reared in standard dark/light conditions (DL-Control) as a comparison. Following the rearing period, brains were sectioned and were stained by immunohistochemistry with tyrosine hydroxide (TH), to define the boundary of locus coeruleus, dull gray; example locus coeruleus boarder shown in Figure 4D), and the p85 fragment of PARP, an *in situ* marker of apoptosis (light gray). Figure 4 shows DREADD activation of retinal cells in constant darkness prevents apoptosis in locus coeruleus. Apoptosis was only observed in DD-control and DD-GFP animals (those without any retinal activation). Apoptosis was not observed in DD-Hm3Dq and DL-Control animals (those with daily retinal activation). The amount of apoptosis was quantified by measuring the intensity of fluorescence that was generated by p85 PARP staining (Figure 4F).

Example 5

PACAP-DREADD Expression in Melanopsin (+) Cells Following Intravitreal Injection (IVI)

In the absence of available melanopsin-based agents to specifically target intrinsically photosensitive retinal ganglion cells (ipRGCs) in non-transgenic models, a pituitary adenylate cyclase-activating polypeptide (PACAP), which is co-expressed in melanopsin expressing retinal cells (Hannibal *J Neurosci.,* 2002), was used to generate a PACAP promoter to drive excitatory DREADDs (PACAP-Hm3Dq) in melanopsin immuno-reactive cells.

Retinae were double stained by fluorescence immunohistochemistry for mCherry (the fluorescent tag fused to the hM3Dq gene, in this construct) and melanopsin. The images from both channels were co-localized. Cells which are overlapping indicate co-expression of hM3Dq and melanopsin. Figure 5A-Figure 5C. Almost all PACAP-Hm3Dq (+) cells are immuno-reactive for melanopsin as assessed by the co-localization, indicating that the PACAP promoter can selectively drive expression of DREADDs in melanopsin cells.

Example 6

DREADD Activation of PACAP Cells Driven by a PACAP-specific Promoter Prevents Depression Associated Weight Loss

Animals that had excitatory PACAP-driven DREADDs expressed in their retinas were designated as DD-PACAP and animals that had non-activating control virus expressed in their retinas were designated as DD-GFP. All animals were housed in total darkness for 24 hours a day, an environmental condition known to lead to depression-like behavior in animals (Gonzalez

and Aston-Jones *PNAS*. 2008). The agonist CNO was administered once per day activating retinal cells in G(q) animals only.

Figure 6 displays weight loss in DD-GFP animals for the first eight weeks of total dark-rearing. As hypothesized, animals with PACAP-driven DREADD (DD-PACAP) activated retinas gained weight. Therefore, the DREADD activation of the PACAP (+) retinal cells significantly reduced the depression-associated behavior induced by constant dark-rearing.

* * * * *

The present invention is not to be limited in scope by the specific embodiments described above. Indeed, various modifications of the invention in addition to those described will become apparent to those skilled in the art from the foregoing description and the accompanying figures. Such modifications may be made without departing from the scope and spirit of the present invention, and are intended to fall within the scope of the appended claims.

All references cited herein are incorporated by reference to the same extent as if each individual publication, database entry (e.g. Genbank sequences or GeneID entries), patent application, or patent, was specifically and individually indicated to be incorporated by reference. This statement of incorporation by reference is intended by Applicants, pursuant to 37 C.F.R. §1.57(b)(1), to relate to each and every individual publication, database entry (e.g. Genbank sequences or GeneID entries), patent application, or patent, each of which is clearly identified in compliance with 37 C.F.R. §1.57(b)(2), even if such citation is not immediately adjacent to a dedicated statement of incorporation by reference. The inclusion of dedicated statements of incorporation by reference, if any, within the specification does not in any way weaken this general statement of incorporation by reference. Citation of the references herein is not intended as an admission that the reference is pertinent prior art, nor does it constitute any admission as to the contents or date of these publications or documents.

Reference to any prior art in the specification is not an acknowledgement or suggestion that this prior art forms part of the common general knowledge in any jurisdiction or that this prior art could reasonably be expected to be combined with any other piece of prior art by a skilled person in the art.

By way of clarification and for avoidance of doubt, as used herein and except where the context requires otherwise, the term "comprise" and variations of the term, such as "comprising", "comprises" and "comprised", are not intended to exclude further additions, components, integers or steps.

CLAIMS

1. A method of treating a disease or disorder of the nervous system in a subject, comprising the steps of:
    a. administering an effective amount of a viral vector to the eye of the subject, wherein the viral vector comprises a promoter comprising SEQ ID NO: 5, a DREADD, and a 3' untranslated region encoded by the nucleotide sequence SEQ ID NO:1 or SEQ ID NO:6;
    b. expressing the DREADD of step (a) prior to administration of an agonist to the DREADD; and
    c. administering to the subject an agonist to the expressed DREADD,
    wherein the disease or disorder of the nervous system is selected from the group consisting of depression, anxiety, sleep disorders, and Alzheimer's disease.
2. Use of a viral vector comprising a promoter comprising SEQ ID NO: 5, a DREADD, and a 3' untranslated region encoded by the nucleotide sequences SEQ ID NO:1 or SEQ ID NO:6, in the manufacture of a medicament for the treatment of a disease or disorder of the nervous system in a subject, wherein the medicament is to be administered to the eye of the subject, and wherein the disease or disorder of the nervous system is selected from the group consisting of depression, anxiety, sleep disorders, and Alzheimer's disease.
3. Use of a viral vector comprising a promoter comprising SEQ ID NO: 5, a DREADD, and a 3' untranslated region encoded by the nucleotide sequences SEQ ID NO:1 or SEQ ID NO:6, in the manufacture of a medicament for treating a disease or disorder of the nervous system in a subject, wherein
    a. the medicament is to be administered to the eye of the subject;
    b. the DREADD of the medicament is to be expressed prior to administration of an agonist to the DREADD.; and
    wherein the disease or disorder of the nervous system is selected from the group consisting of depression, anxiety, sleep disorders, and Alzheimer's disease.
4. The method of claim 1, or the use of claim 2 or 3, wherein the nucleotide sequence is SEQ ID NO:1.
5. The method claim 1, or the use of claim 2 or 3, wherein the nucleotide sequence is SEQ ID NO:6.

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6. The method or use of any one of claims 1 to 5, wherein the viral vector is an adeno-associated viral vector (AAV) selected from the group consisting of: AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV11, AAV12, and hybrids thereof.
7. The method of any one of claims 1, 4 to 6, or the use of any one of claims 3 to 6, wherein the agonist is clozapine N-oxide, DREADD agonist 21, salvinorin B, clozapine, olanzapine, or perlapine.
8. The method of any one of claims 1 or 4 to 7, wherein the agonist is administered systemically or to the eye, or the use of any one of claims 3 to 7, wherein the agonist is to be administered systemically or to the eye.
9. The method of any one of claims 1 or 4 to 8, further comprising administering at least one additional therapeutic agent for treatment of the disease or disorder of the nervous system.
10. The use of any one of claims 2 to 8, wherein the medicament is to be administered in combination with at least one additional therapeutic agent for treatment of the disease or disorder of the nervous system.
11. The use of any one of claims 2 to 8, wherein the medicament further comprises at least one additional therapeutic agent for treatment of the disease or disorder of the nervous system.
12. The method of claim 9 or the use of claim 10 or 11, wherein the at least one additional therapeutic agent is a neurological drug selected from the group consisting of acamprosate, agomelatine, alimemazine, alprazolam, amantadine, amfetamine, amisulpride, amitriptyline, amobarbital, amoxapine, apomorphine, apomorphine, aripiprazole, asenapine, atomoxetine, atropine, baclofen, benperidol, benztropine, biperiden, bromazepam, bromocriptine, bromperidol, brotizolam, buprenorphine, bupropion, buspirone, butobarbital, cabergoline, carbamazepine, chloral hydrate, chlordiazepoxide, chlorpheniramine, chlorpromazine, chlorprothixene, citalopram, clobazam, clomethiazole, clomipramine, clonazepam, clonidine, clorazepate, clozapine, cyclobarbital, cyproheptadine, cytisine, desipramine, desvenlafaxine, dexamfetamine, dexmethylphenidate, dextromethorphan, diazepam, dicyclomine dimenhydrinate, diphenhydramine, disulfiram, divalproex sodium, donepezil, doxacurium, doxepin, doxylamine, duloxetine, edaravone, enanthate, escitalopram, estazolam, eszopiclone, ethosuximide, flunitrazepam, fluoxetine, flupenthixol, fluphenazine, flurazepam, fluspirilen, fluvoxamine, gabapentin, galantamine, glutethimide, glycopyrrolate, guanfacine, haloperidol, hexamethonium, hydrochloride, hydroxyzine, iloperidone,

imipramine, ipratropium, lamotrigine, levetiracetam, levodopa, levomepromazine, levomilnacipran, lisdexamfetamine, lisuride, lithium salts, loprazolam, lorazepam, lormetazepam, mecamylamine, melatonin, melperone, memantine, meprobamate, metamfetamine, methadone, methylphenidate, mianserin, midazolam, mirtazapine, moclobemide, modafinil, modecate, motherwort, nalmefene, naltrexone, niaprazine, nimetazepam, nitrazepam, nortriptyline, olanzapine, omca, ondansetron, orphenadrine, oxazepam, oxcarbazepine, oxitropium, oxybutynin, paliperidone, paroxetine, penfluridol, pentobarbital, perazine, pergolide, pericyazine, perphenazine, phenazepam, phenelzine phenobarbital, phenytoin, pimozide, piribedil, pramipexole, pregabalin, prolixin decanoate, promethazine, propantheline bromide, prothipendyl, protriptyline, quazepam, quetiapine, ramelteon, rasagiline, reboxetine, remacemide, reserpine, riluzole, risperidone, rivastigmine, ropinirole, rotigotine, rubidium chloride, safinamide, scopolamine, secobarbital, sediten, selecten, selegiline, selegiline, sertindole, sertraline, sertraline, sevinol, sinqualone enantat, siqualone, sirtal, sodium oxybate, sodium valproate, solifenacin, stazepine, stelazine, sulpiride, suvorexant, tacrine, tegretol, telesmin, temazepam, terfluzine, tetrabenazine, thioridazine, thiothixene, tianeptine, timonil, tiotropium, tizanidine, tofisopam, tolcapone, tolterodine, topiramate, trancin, tranylcypromine, trazodone, triazolam, trifluoperaz, trifluoperazine, triftazin, trihexyphenidyl, trimipramine, tropicamide, tubocurarine, valerian, valproate, valproic acid, varenicline, venlafaxine, vilazodone, vortioxetine, zaleplon, ziprasidone, zolpidem, zopiclone, zotepine, zuclopenthixol, and combinations thereof.

13. A method of delivery of a DREADD to the retina of a subject to control activation of the Photic Regulation of Arousal and Mood (PRAM) pathway comprising:
    a. administering an effective amount of a viral vector to the eye of the subject, wherein the viral vector comprises SEQ ID NO:1 or SEQ ID NO:6 encoding a promoter comprising SEQ ID NO:5, a DREADD, and a 3' untranslated region;
    b. expressing the DREADD of step (a) prior to administration of an agonist to the DREADD; and
    c. administering to the subject an agonist to the expressed DREADD to control activation of the PRAM pathway.

14. A kit comprising a viral vector for administration to the eye of a subject, wherein the viral vector comprises a promoter comprising SEQ ID NO: 5, a DREADD, and a 3' untranslated

region encoded by the nucleotide sequence selected from the group consisting of SEQ ID NO:1 or SEQ ID NO:6, and an agonist to the DREADD, when used in the method or use of any one of claims 1 to 13.

Figure 1

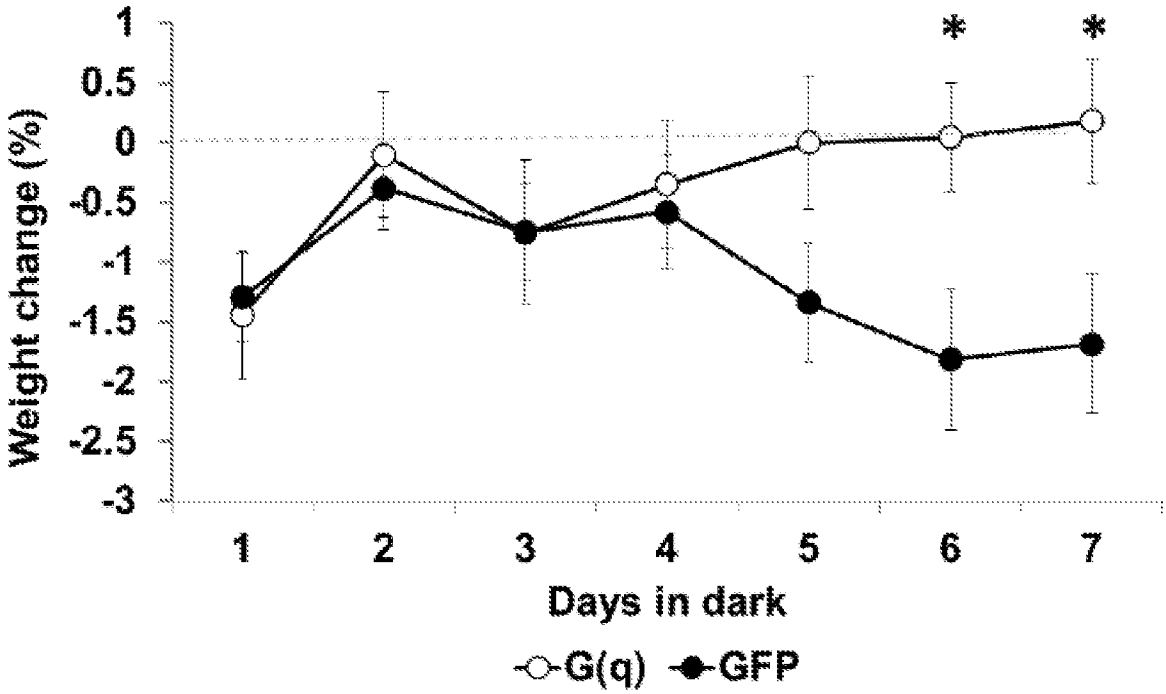


Figure 2

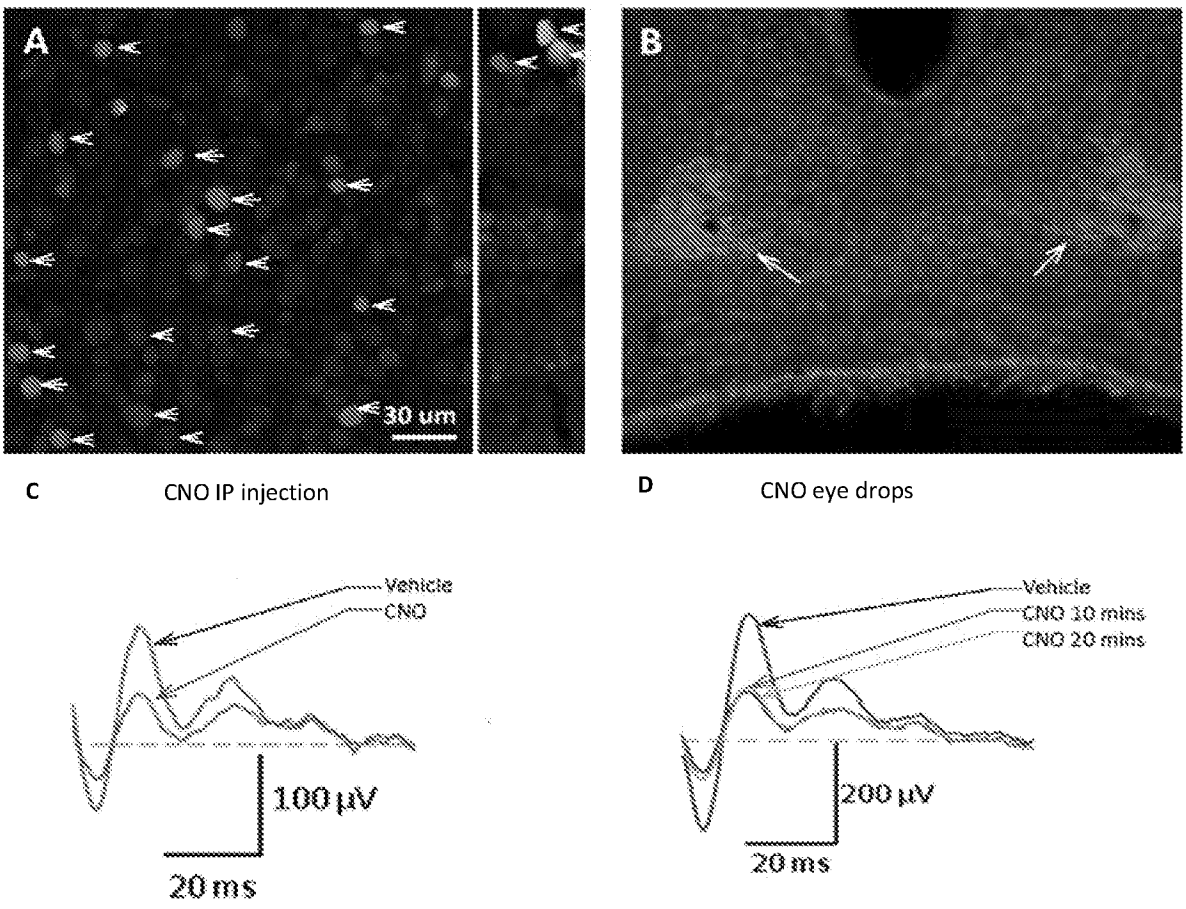

A
30 um
B
C
CNO IP injection
D
CNO eye drops
Vehicle
CNO
100 µV
20 ms
Vehicle
CNO 10 mins
CNO 20 mins
200 µV
20 ms

Figure 3

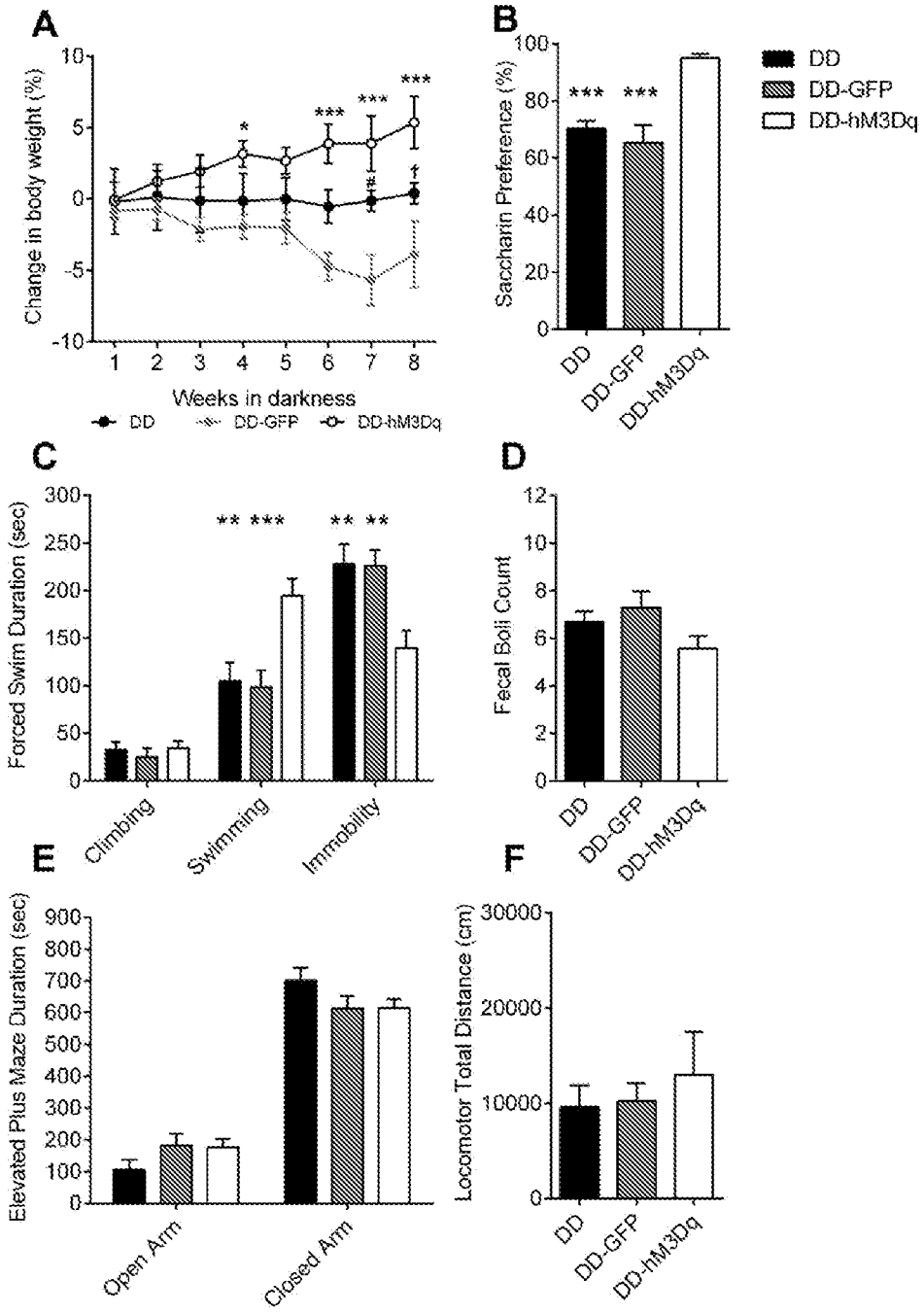
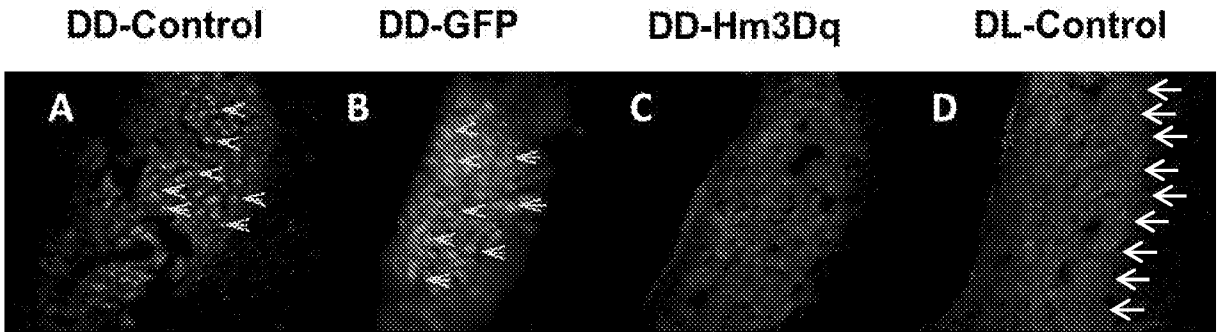

A
Change in body weight (%)
Weeks in darkness
DD
DD-GFP
DD-hM3Dq
B
Saccharin Preference (%)
C
Forced Swim Duration (sec)
Climbing
Swimming
Immobility
D
Fecal Boli Count
E
Elevated Plus Maze Duration (sec)
Open Arm
Closed Arm
F
Locomotor Total Distance (cm)

Figure 4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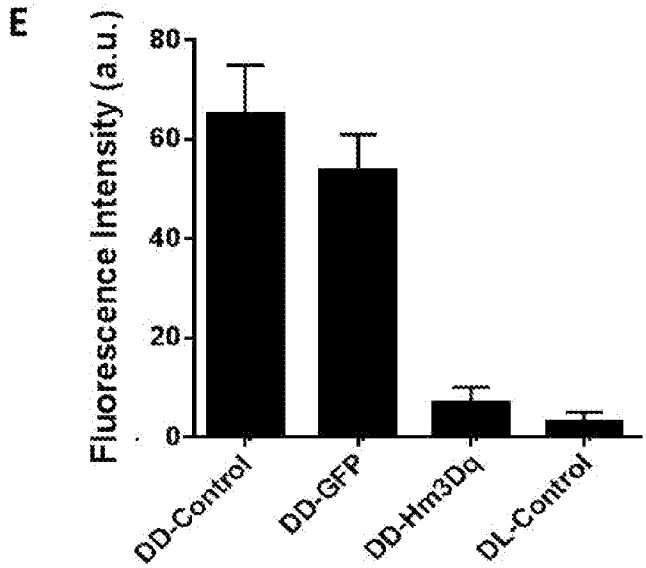
DD-GFP
DD-Hm3Dq
DL-Control
A
B
C
D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60
40
20
0
DD-Control
DD-GFP
DD-Hm3Dq
DL-Control

Figure 5

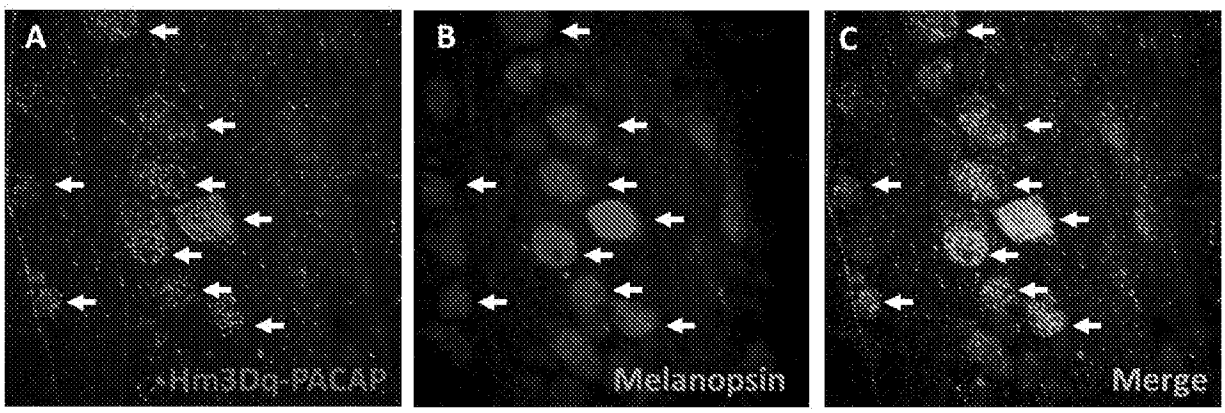
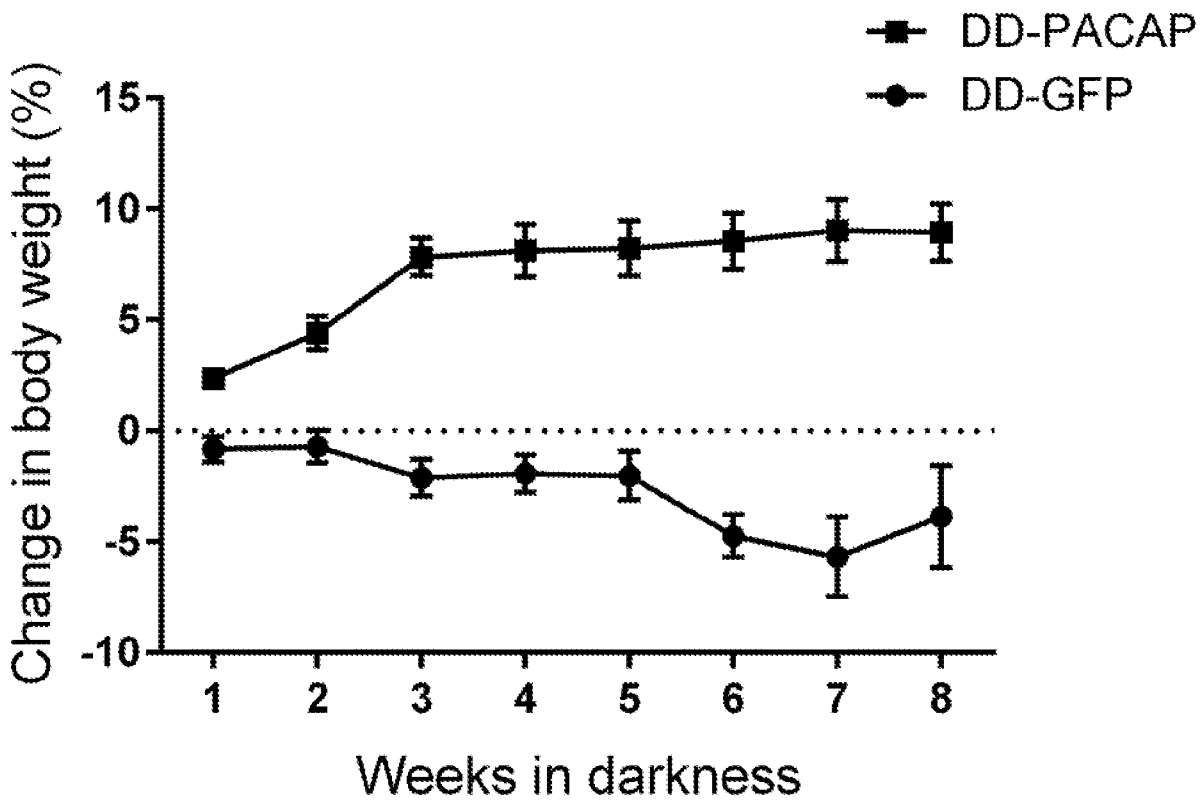


Figure 6


DD-PACAP
DD-GFP
Change in body weight (%)
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5
0
-5
-10
1
2
3
4
5
6
7
8
Weeks in darkness

SEQUENCE LISTING

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<120> METHODS AND COMPOSITIONS FOR TREATING DISEASES AND DISORDERS OF THE NERVOUS SYSTEM

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gctaagactt ctgacgtcaa ctcctcagtg ggtaagagca cggccactct acctctgtcc 2460

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cggaaaagga tgtccctggt caaggagaag aaagcggccc agaccctcag tgcgatcttg 2580

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gacagctgca tacccaaaac cttttggaat ctgggctact ggctgtgcta catcaacagc 2700

accgtgaacc ccgtgtgcta tgctctgtgc aacaaaacat tcagaaccac tttcaagatg 2760

ctgctgctgt gccagtgtga caaaaaaaag aggcgcaagc agcagtacca gcagagacag 2820

tcggtcattt ttcacaagcg cgcacccgag caggccttga aggatccccc ggtcgccacc 2880

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