

TARGETED GENE THERAPY TO TREAT NEUROLOGICAL DISEASES

CROSS-REFERENCE TO RELATED APPLICATIONS

5 This application claims the benefit of the filing date of U.S. application No. 62/881,540, filed on August 1, 2019, the disclosure of which is incorporated by reference herein.

BACKGROUND

10 Gene therapy has shown great promise in human patients for treating neurological diseases. One advantage of invasive injection of gene therapy agents is the focal targeting of relevant brain regions to prevent off target effects that often limit efficacy and cause side effects from drugs delivered throughout the brain. It is increasingly recognized, however, that targeting specific circuits
15 can not only provide a greater level of specificity compared with targeting brain regions, but that resulting behaviors can differ when a specific circuit underlying that behavior is manipulated in isolation. One way to limit expression to specific subpopulations of neurons is to use cell-type specific promoters, but these are often unreliable and still do not provide anatomic circuit specificity but rather
20 restrict expression to all neurons expressing the gene driven by the chosen promoter. Therefore, anatomic targeting methods are desirable to manipulate populations of neurons which project from one brain region to regulate neurons in another brain region, referred to here as circuits.

 One example of this approach is optogenetics. In this method, a gene for
25 a light-sensitive ion channel is delivered to a brain region, usually by a viral vector, and then the ion channel is expressed in all transduced neurons within a brain region capable of supporting expression from the given promoter. A light probe is then placed within a target region so that the only neurons which will be activated are those which received the gene into the cell bodies in the first brain
30 target and light to the axons in the second brain target. However, this requires an implanted light probe and cannot be regulated non-invasively, and the safety of chronic light in the brain remains unknown.

 Accordingly, there is a need for a methodology which can permit modulation of specific neuronal circuits through non-invasive means. There is

also a need for a methodology to non-invasively target gene delivery exclusively to specific neuronal circuits.

SUMMARY

5 A method for targeting a specific population of neurons in a mammal, e.g., a human, is provided. In one embodiment, the method comprises delivery (administration) of a first viral vector with a conditionally activatable (non-functional until activated) gene or gene product to a mammalian structure to be modulated, e.g., which contains or is in proximity to axons from regulatory
10 neurons, and delivery of a second viral vector expressing a gene, the product of which can activate the gene in the first viral vector, e.g., the second viral vector may be delivered to a different region containing a subset of the cell bodies that send the axons to the structure to be modulated. In one embodiment, the method comprises delivery of a viral vector with a conditionally activatable gene or gene
15 product to cell bodies that send the axons to a structure to be modulated and delivery of a second vector expressing a gene, the product of which can activate the gene in the first viral vector, e.g., to the structure to be modulated. In one embodiment, the method comprises delivery of a first viral vector with a conditionally activatable gene or gene product, which first vector is capable of
20 retrograde uptake into neurons, and delivery of a second vector expressing a gene, the product of which can activate the first viral vector. In one embodiment, the method comprises delivery of a first viral vector with a conditionally activatable gene or gene product, and delivery of a second vector expressing a gene, the product of which can activate the first viral vector, which second
25 vector is capable of retrograde uptake into neurons. , to a different region containing a subset of the cell bodies that send the axons to the structure to be modulated. In one embodiment, the structure to be modulated is a peripheral organ or a brain region. In one embodiment, the viral vector is an adeno-associated virus, adenovirus, canine adenovirus, herpes simplex virus, or
30 lentivirus vector, e.g., a retrograde form of adeno-associated virus, lentivirus or canine adenovirus. Molecules that provide for retrograde forms of vectors are known to the art and include but are not limited to native viral proteins, such as HSV protein, rabies virus G, glycoprotein type C, VSV G, B19G, pseudorabies virus protein, AAV capsid protein, and dynein. In one embodiment, the first

vector contains recognition sites capable of recombination and activation of the gene, e.g., a therapeutic gene, in response to a recombinase delivered by the second vector. In one embodiment, the recombinase is Cre or Flp. In one embodiment, a recombinase enzyme is conditionally activated by an exogenous
5 chemical or stimulus.

In one embodiment, the first vector contains a gene that does not express a functional protein autonomously, and the second vector expresses a protein that is capable of activating gene expression or protein function from the first gene. In one embodiment, the second vector expresses regulatable or
10 autonomously active transcription factors capable of transactivating expression of the gene, e.g., encoding a therapeutic gene product, from the first vector.

In one embodiment, the viral vector is delivered via direct infusion into the organ or brain region. In one embodiment, the viral vector is delivered through focal disruption of the blood-brain barrier using focused ultrasound. In
15 one embodiment, the viral vector is delivered to one brain region in a single treatment, followed by delivery of a second viral vector to a second brain region in a second treatment. In one embodiment, the two treatments are separated by at least 24, 36 or 72 hours or more. In one embodiment, the two treatments are sequential, e.g., occur on the same day. In one embodiment, the two treatments
20 are conducted simultaneously. In one embodiment, the two treatments are separated by 1, 2 or 3 weeks or more.

Further provided is a system or a kit comprising a first vector with a non-functional (conditionally activatable) gene or gene product, and a second vector expressing a gene, the product of which can activate the gene in the first vector,
25 wherein optionally one of the vectors is capable of retrograde uptake in neurons. In one embodiment, the vectors are both viral vectors, e.g., AAV vectors that may differ in serotype. In one embodiment, the first vector encodes an ion channel protein. In one embodiment, the first vector encodes a G coupled protein receptor ion channel protein. In one embodiment, the first vector
30 encodes a transcription factor.

In one embodiment, one vector comprises an open reading frame for a selected (first) gene product and the other vector comprises an open reading frame for a selected (second) gene product. Expression of one of the gene products is conditionally activatable by the other gene product. For example,

one of the open reading frames is flanked by recombination sites for a recombinase that is encoded by the other vector. In one embodiment, one of the vectors is a recombinant viral vector, e.g., rAAV vector. In one embodiment, both of the vectors are recombinant viral vectors. In one embodiment, when both
 5 of the vectors are recombinant viral vectors, one is a retrograde viral vector.

In one embodiment, non-invasive delivery of two vectors is employed. In one embodiment, the delivery of the vectors is sequential. For example, one (first) vector is systemically delivered, e.g., via injection, and focused ultrasound is used to target that vector to a specific site in the central nervous system. Then
 10 the other (second) vector is systemically delivered, e.g., via injection, and focused ultrasound is used to target that vector to a specific (e.g., different) site in the central nervous system. In one embodiment, after systemically delivering, e.g., via injection, the second vector, focused ultrasound is used to target that vector to the same site as the first vector.

15 In one embodiment, invasive delivery of two vectors is employed. In one embodiment, the delivery of the vectors is on the same day. In one embodiment, the delivery of the vectors is sequential, e.g., separated by hours, days or weeks. In one embodiment, the vectors are delivered to the same site. In one embodiment, the vectors are delivered to different sites.

20 In one embodiment, non-invasive delivery of one vector and invasive delivery of another vector are employed. In one embodiment, the delivery of the vectors is on the same day. In one embodiment, the delivery of the vectors is sequential. In one embodiment, the vectors are delivered to the same site. In one embodiment, the vectors are delivered to different sites.

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BRIEF DESCRIPTION OF FIGURES

Figure 1. Sagittal section through rat brain. Viral vectors are delivered as depicted through either MRgFUS or stereotactic direct injection.

30 Figure 2. Stereotactic injection of AAV into rat brain. Immunostaining for mCherry (reporter gene), GFP (reporter gene), TH (tyrosine hydroxylase – dopaminergic neurons marker) and DAPI (nuclear marker) following unilateral and ipsilateral administration of AAV1/2.DIO.hM3Dq.mCherry into striatum and RetroAAV1/2.Cre.GFP into substantia nigra.

Figure 3. Stereotactic injection of AAV into rat brain. Immunostaining for mCherry (reporter gene), GFP (reporter gene), TH (tyrosine hydroxylase – dopaminergic neurons marker) and DAPI (nuclear marker) following unilateral and ipsilateral administration of AAV1/2.DIO.hM3Dq.mCherry into striatum and RetroAAV1/2.GFP into substantia nigra.

Figure 4. MRgFUS-mediated delivery of AAV into rat brain: Immunostaining for mCherry (reporter gene), GFP (reporter gene), TH (tyrosine hydroxylase–dopaminergic neurons marker) and DAPI (nuclear marker) following unilateral and ipsilateral administration of AAV1/2.DIO.hM3Dq.mCherry into striatum and RetroAAV1/2.Cre.GFP into substantia nigra.

Figure 5. MRgFUS-mediated delivery of AAV into rat brain: Immunostaining for mCherry (reporter gene), GFP (reporter gene), TH (tyrosine hydroxylase – dopaminergic neurons marker) and DAPI (nuclear marker) following unilateral and ipsilateral administration of AAV1/2.DIO.hM3Dq.mCherry into striatum and RetroAAV1/2.GFP into substantia nigra.

DETAILED DESCRIPTION

Definitions

A “vector” refers to a macromolecule or association of macromolecules that comprises or associates with a polynucleotide, and which can be used to mediate delivery of the polynucleotide to a cell, either *in vitro* or *in vivo*. Illustrative vectors include, for example, plasmids, viral vectors, liposomes and other gene delivery vehicles. The polynucleotide to be delivered, sometimes referred to as a “target polynucleotide” or “transgene,” may comprise a coding sequence of interest in gene therapy (such as a gene encoding a protein of therapeutic interest), a coding sequence of interest in vaccine development (such as a polynucleotide expressing a protein, polypeptide or peptide suitable for eliciting an immune response in a mammal), and/or a selectable or detectable marker.

“Transduction,” “transfection,” “transformation” or “transducing” as used herein, are terms referring to a process for the introduction of an exogenous polynucleotide into a host cell leading to expression of the polynucleotide, e.g.,

the transgene in the cell, and includes the use of recombinant virus to introduce the exogenous polynucleotide to the host cell. Transduction, transfection or transformation of a polynucleotide in a cell may be determined by methods well known to the art including, but not limited to, protein expression (including
5 steady state levels), e.g., by ELISA, flow cytometry and Western blot, measurement of DNA and RNA by hybridization assays, e.g., Northern blots, Southern blots and gel shift mobility assays. Methods used for the introduction of the exogenous polynucleotide include well-known techniques such as viral infection or transfection, lipofection, transformation and electroporation, as well
10 as other non-viral gene delivery techniques. The introduced polynucleotide may be stably or transiently maintained in the host cell.

“Gene delivery” refers to the introduction of an exogenous polynucleotide into a cell for gene transfer, and may encompass targeting, binding, uptake, transport, localization, replicon integration and expression.

15 “Gene transfer” refers to the introduction of an exogenous polynucleotide into a cell which may encompass targeting, binding, uptake, transport, localization and replicon integration, but is distinct from and does not imply subsequent expression of the gene.

“Gene expression” or “expression” refers to the process of gene
20 transcription, translation, and post-translational modification.

An “infectious” virus or viral particle is one that comprises a polynucleotide component which it is capable of delivering into a cell for which the viral species is trophic. The term does not necessarily imply any replication capacity of the virus.

25 The term “polynucleotide” refers to a polymeric form of nucleotides of any length, including deoxyribonucleotides or ribonucleotides, or analogs thereof. A polynucleotide may comprise modified nucleotides, such as methylated or capped nucleotides and nucleotide analogs, and may be interrupted by non-nucleotide components. If present, modifications to the
30 nucleotide structure may be imparted before or after assembly of the polymer. The term polynucleotide, as used herein, refers interchangeably to double- and single-stranded molecules. Unless otherwise specified or required, any embodiment described herein that is a polynucleotide encompasses both the

double-stranded form and each of two complementary single-stranded forms known or predicted to make up the double-stranded form.

An "isolated" polynucleotide, e.g., plasmid, virus, polypeptide or other substance refers to a preparation of the substance devoid of at least some of the other components that may also be present where the substance or a similar substance naturally occurs or is initially prepared from. Thus, for example, an isolated substance may be prepared by using a purification technique to enrich it from a source mixture. Isolated nucleic acid, peptide or polypeptide is present in a form or setting that is different from that in which it is found in nature. For example, a given DNA sequence (e.g., a gene) is found on the host cell chromosome in proximity to neighboring genes; RNA sequences, such as a specific mRNA sequence encoding a specific protein, are found in the cell as a mixture with numerous other mRNAs that encode a multitude of proteins. The isolated nucleic acid molecule may be present in single-stranded or double-stranded form. When an isolated nucleic acid molecule is to be utilized to express a protein, the molecule will contain at a minimum the sense or coding strand (i.e., the molecule may single-stranded), but may contain both the sense and anti-sense strands (i.e., the molecule may be double-stranded). Enrichment can be measured on an absolute basis, such as weight per volume of solution, or it can be measured in relation to a second, potentially interfering substance present in the source mixture. Increasing enrichments of the embodiments may be preferred. Thus, for example, a 2-fold enrichment, 10-fold enrichment, 100-fold enrichment, or a 1000-fold enrichment.

A "transcriptional regulatory sequence" refers to a genomic region that controls the transcription of a gene or coding sequence to which it is operably linked. Transcriptional regulatory sequences of use in the present disclosure generally include at least one transcriptional promoter and may also include one or more enhancers and/or terminators of transcription.

"Operably linked" refers to an arrangement of two or more components, wherein the components so described are in a relationship permitting them to function in a coordinated manner. By way of illustration, a transcriptional regulatory sequence or a promoter is operably linked to a coding sequence if the TRS or promoter promotes transcription of the coding sequence. An operably

linked TRS is generally joined in *cis* with the coding sequence, but it is not necessarily directly adjacent to it.

“Heterologous” means derived from a genotypically distinct entity from the entity to which it is compared. For example, a polynucleotide introduced by genetic engineering techniques into a different cell type is a heterologous polynucleotide (and, when expressed, can encode a heterologous polypeptide). Similarly, a transcriptional regulatory element such as a promoter that is removed from its native coding sequence and operably linked to a different coding sequence is a heterologous transcriptional regulatory element.

10 A “terminator” refers to a polynucleotide sequence that tends to diminish or prevent read-through transcription (i.e., it diminishes or prevent transcription originating on one side of the terminator from continuing through to the other side of the terminator). The degree to which transcription is disrupted is typically a function of the base sequence and/or the length of the terminator sequence. In particular, as is well known in numerous molecular biological systems, particular DNA sequences, generally referred to as “transcriptional termination sequences” are specific sequences that tend to disrupt read-through transcription by RNA polymerase, presumably by causing the RNA polymerase molecule to stop and/or disengage from the DNA being transcribed. Typical
15 example of such sequence-specific terminators include polyadenylation (“polyA”) sequences, e.g., SV40 polyA. In addition to or in place of such sequence-specific terminators, insertions of relatively long DNA sequences between a promoter and a coding region also tend to disrupt transcription of the coding region, generally in proportion to the length of the intervening sequence. This effect presumably arises because there is always some tendency for an RNA polymerase molecule to become disengaged from the DNA being transcribed, and increasing the length of the sequence to be traversed before reaching the coding region would generally increase the likelihood that disengagement would occur before transcription of the coding region was
20 completed or possibly even initiated. Terminators may thus prevent transcription from only one direction (“uni-directional” terminators) or from both directions (“bi-directional” terminators), and may be comprised of sequence-specific termination sequences or sequence-non-specific terminators or both. A variety of such terminator sequences are known in the art; and

illustrative uses of such sequences within the context of the present disclosure are provided below.

“Host cells,” “cell lines,” “cell cultures,” “packaging cell line” and other such terms denote higher eukaryotic cells, such as mammalian cells including human cells, useful in the present disclosure, e.g., to produce recombinant virus or recombinant fusion polypeptide. These cells include the progeny of the original cell that was transduced. It is understood that the progeny of a single cell may not necessarily be completely identical (in morphology or in genomic complement) to the original parent cell.

“Recombinant,” as applied to a polynucleotide means that the polynucleotide is the product of various combinations of cloning, restriction and/or ligation steps, and other procedures that result in a construct that is distinct from a polynucleotide found in nature. A recombinant virus is a viral particle comprising a recombinant polynucleotide. The terms respectively include replicates of the original polynucleotide construct and progeny of the original virus construct.

A “control element” or “control sequence” is a nucleotide sequence involved in an interaction of molecules that contributes to the functional regulation of a polynucleotide, including replication, duplication, transcription, splicing, translation, or degradation of the polynucleotide. The regulation may affect the frequency, speed, or specificity of the process, and may be enhancing or inhibitory in nature. Control elements known in the art include, for example, transcriptional regulatory sequences such as promoters and enhancers. A promoter is a DNA region capable under certain conditions of binding RNA polymerase and initiating transcription of a coding region usually located downstream (in the 3' direction) from the promoter. Promoters include AAV promoters, e.g., P5, P19, P40 and AAV ITR promoters, as well as heterologous promoters.

An “expression vector” is a vector comprising a region which encodes a gene product of interest, and is used for effecting the expression of the gene product in an intended target cell. An expression vector also comprises control elements operatively linked to the encoding region to facilitate expression of the protein in the target. The combination of control elements and a gene or genes to which they are operably linked for expression is sometimes referred to as an

“expression cassette,” a large number of which are known and available in the art or can be readily constructed from components that are available in the art.

The terms “polypeptide” and “protein” are used interchangeably herein to refer to polymers of amino acids of any length. The terms also encompass an amino acid polymer that has been modified; for example, disulfide bond
5 formation, glycosylation, acetylation, phosphorylation, lipidation, or conjugation with a labeling component.

The term “exogenous,” when used in relation to a protein, gene, nucleic acid, or polynucleotide in a cell or organism refers to a protein, gene, nucleic acid, or polynucleotide which has been introduced into the cell or organism by
10 artificial or natural means. An exogenous nucleic acid may be from a different organism or cell, or it may be one or more additional copies of a nucleic acid which occurs naturally within the organism or cell. By way of a non-limiting example, an exogenous nucleic acid is in a chromosomal location different from
15 that of natural cells, or is otherwise flanked by a different nucleic acid sequence than that found in nature, e.g., an expression cassette which links a promoter from one gene to an open reading frame for a gene product from a different gene.

“Transformed” or “transgenic” is used herein to include any host cell or
20 cell line, which has been altered or augmented by the presence of at least one recombinant DNA sequence. The host cells are typically produced by transfection with a DNA sequence in a plasmid expression vector, as an isolated linear DNA sequence, or infection with a recombinant viral vector.

The term “sequence homology” means the proportion of base matches
25 between two nucleic acid sequences or the proportion amino acid matches between two amino acid sequences. When sequence homology is expressed as a percentage, e.g., 50%, the percentage denotes the proportion of matches over the length of a selected sequence that is compared to some other sequence. Gaps (in either of the two sequences) are permitted to maximize matching; gap lengths of
30 15 bases or less are usually used, 6 bases or less, or 2 bases or less. When using oligonucleotides as probes or treatments, the sequence homology between the target nucleic acid and the oligonucleotide sequence is generally not less than 17 target base matches out of 20 possible oligonucleotide base pair matches (85%);

not less than 9 matches out of 10 possible base pair matches (90%), or not less than 19 matches out of 20 possible base pair matches (95%).

Two amino acid sequences are homologous if there is a partial or complete identity between their sequences. For example, 85% homology means
5 that 85% of the amino acids are identical when the two sequences are aligned for maximum matching. Gaps (in either of the two sequences being matched) are allowed in maximizing matching; gap lengths of 5 or less or 2 or less.

Alternatively, two protein sequences (or polypeptide sequences derived from them of at least 30 amino acids in length) are homologous, as this term is used
10 herein, if they have an alignment score of at more than 5 (in standard deviation units) using the program ALIGN with the mutation data matrix and a gap penalty of 6 or greater. The two sequences or parts thereof are more homologous if their amino acids are greater than or equal to 50% identical when optimally aligned using the ALIGN program.

15 The term “corresponds to” is used herein to mean that a polynucleotide sequence is structurally related to all or a portion of a reference polynucleotide sequence, or that a polypeptide sequence is structurally related to all or a portion of a reference polypeptide sequence, e.g., they have at least 80%, 85%, 90%, 95% or more, e.g., 99% or 100%, sequence identity. In contradistinction, the
20 term “complementary to” is used herein to mean that the complementary sequence is homologous to all or a portion of a reference polynucleotide sequence. For illustration, the nucleotide sequence “TATAC” corresponds to a reference sequence “TATAC” and is complementary to a reference sequence “GTATA”.

25 The term “sequence identity” means that two polynucleotide sequences are identical (i.e., on a nucleotide-by-nucleotide basis) over the window of comparison. The term “percentage of sequence identity” means that two polynucleotide sequences are identical (i.e., on a nucleotide-by-nucleotide basis) over the window of comparison. The term “percentage of sequence identity” is
30 calculated by comparing two optimally aligned sequences over the window of comparison, determining the number of positions at which the identical nucleic acid base (e.g., A, T, C, G, U, or I) occurs in both sequences to yield the number of matched positions, dividing the number of matched positions by the total number of positions in the window of comparison (i.e., the window size), and

5 multiplying the result by 100 to yield the percentage of sequence identity. The terms "substantial identity" as used herein denote a characteristic of a polynucleotide sequence, wherein the polynucleotide comprises a sequence that has at least 85 percent sequence identity, at least 90 to 95 percent sequence identity, more usually at least 99 percent sequence identity as compared to a reference sequence over a comparison window of at least 20 nucleotide positions, frequently over a window of at least 20-50 nucleotides, wherein the percentage of sequence identity is calculated by comparing the reference sequence to the polynucleotide sequence which may include deletions or additions which total 20 percent or less of the reference sequence over the window of comparison.

"Conservative" amino acid substitutions are, for example, aspartic-glutamic as polar acidic amino acids; lysine/arginine/histidine as polar basic amino acids; leucine/isoleucine/methionine/valine/alanine/glycine/proline as non-polar or hydrophobic amino acids; serine/ threonine as polar or uncharged hydrophilic amino acids. Conservative amino acid substitution also includes groupings based on side chains. For example, a group of amino acids having aliphatic side chains is glycine, alanine, valine, leucine, and isoleucine; a group of amino acids having aliphatic-hydroxyl side chains is serine and threonine; a group of amino acids having amide-containing side chains is asparagine and glutamine; a group of amino acids having aromatic side chains is phenylalanine, tyrosine, and tryptophan; a group of amino acids having basic side chains is lysine, arginine, and histidine; and a group of amino acids having sulfur-containing side chains is cysteine and methionine. For example, it is reasonable to expect that replacement of a leucine with an isoleucine or valine, an aspartate with a glutamate, a threonine with a serine, or a similar replacement of an amino acid with a structurally related amino acid will not have a major effect on the properties of the resulting polypeptide. Whether an amino acid change results in a functional polypeptide can readily be determined by assaying the specific activity of the polypeptide. Naturally occurring residues are divided into groups based on common side-chain properties: (1) hydrophobic: norleucine, met, ala, val, leu, ile; (2) neutral hydrophilic: cys, ser, thr; (3) acidic: asp, glu; (4) basic: asn, gln, his, lys, arg; (5) residues that influence chain orientation: gly, pro; and (6) aromatic; trp, tyr, phe.

The disclosure also envisions polypeptides with non-conservative substitutions. Non-conservative substitutions entail exchanging a member of one of the classes described above for another.

Gene Transfer Vectors

5 The disclosure provides a gene transfer vector, e.g., a viral gene transfer vector, useful to deliver genes to neurons or nerve fibers. Various aspects of the gene transfer vector and method are discussed below. Accordingly, any combination of parameters can be used according to the gene transfer vector and the method.

10 A “gene transfer vector” is any molecule or composition that has the ability to carry a heterologous nucleic acid sequence into a suitable host cell where synthesis of the encoded protein takes place. Typically, a gene transfer vector is a nucleic acid molecule that has been engineered, using recombinant DNA techniques that are known in the art, to incorporate the heterologous
15 nucleic acid sequence. Desirably, the gene transfer vector is comprised of DNA. Examples of suitable DNA-based gene transfer vectors include plasmids and viral vectors. However, gene transfer vectors that are not based on nucleic acids, such as liposomes, are also known and used in the art. The gene transfer vector can be based on a single type of nucleic acid (e.g., a plasmid) or non-nucleic acid
20 molecule (e.g., a lipid or a polymer). The gene transfer vector can be integrated into the host cell genome, or can be present in the host cell in the form of an episome.

 In one embodiment, the gene transfer vector is a viral vector. Suitable viral vectors include, for example, retroviral vectors, herpes simplex virus
25 (HSV)-based vectors, parvovirus-based vectors, e.g., adeno-associated virus (AAV)-based vectors, AAV-adenoviral chimeric vectors, and adenovirus-based vectors. These viral vectors can be prepared using standard recombinant DNA techniques described in, for example, Sambrook et al., *Molecular Cloning, a Laboratory Manual*, 3rd edition, Cold Spring Harbor Press, Cold Spring Harbor,
30 N.Y. (2001), and Ausubel et al., *Current Protocols in Molecular Biology*, Greene Publishing Associates and John Wiley & Sons, New York, N.Y. (1994).

 In an embodiment, the disclosure provides an adeno-associated virus (AAV) vector. The AAV vector may include a gene to be expressed and additional components that do not materially affect the AAV vector (e.g., genetic

elements such as poly(A) sequences or restriction enzyme sites that facilitate manipulation of the vector *in vitro*). Adeno-associated virus is a member of the Parvoviridae family and comprises a linear, single-stranded DNA genome of less than about 5,000 nucleotides. AAV requires co-infection with a helper virus
5 (i.e., an adenovirus or a herpes virus), or expression of helper genes, for efficient replication. AAV vectors used for administration of therapeutic nucleic acids typically have approximately 96% of the parental genome deleted, such that only the terminal repeats (ITRs), which contain recognition signals for DNA
10 replication and packaging, remain. This eliminates immunologic or toxic side effects due to expression of viral genes. In addition, delivering specific AAV proteins to producing cells enables integration of the AAV vector comprising AAV ITRs into a specific region of the cellular genome, if desired (see, e.g., U.S. Patents 6,342,390 and 6,821,511). Host cells comprising an integrated AAV genome show no change in cell growth or morphology (see, for example,
15 U.S. Patent 4,797,368).

The AAV ITRs flank the unique coding nucleotide sequences for the non-structural replication (Rep) proteins and the structural capsid (Cap) proteins (also known as virion proteins (VPs)). The terminal 145 nucleotides are self-complementary and are organized so that an energetically stable intramolecular
20 duplex forming a T-shaped hairpin may be formed. These hairpin structures function as an origin for viral DNA replication by serving as primers for the cellular DNA polymerase complex. The Rep genes encode the Rep proteins Rep78, Rep68, Rep52, and Rep40. Rep78 and Rep68 are transcribed from the p5 promoter, and Rep 52 and Rep40 are transcribed from the p19 promoter. The
25 Rep78 and Rep68 proteins are multifunctional DNA binding proteins that perform helicase and nickase functions during productive replication to allow for the resolution of AAV termini (see, e.g., Im et al., *Cell*, 61:447 (1990)). These proteins also regulate transcription from endogenous AAV promoters and promoters within helper viruses (see, e.g., Pereira et al., *J. Virol.*, 71:1079
30 (1997)). The other Rep proteins modify the function of Rep78 and Rep68. The cap genes encode the capsid proteins VP1, VP2, and VP3. The cap genes are transcribed from the p40 promoter.

The AAV vector may be generated using any AAV serotype known in the art. Several AAV serotypes and over 100 AAV variants have been isolated

from adenovirus stocks or from human or nonhuman primate tissues (reviewed in, e.g., Wu et al., Molecular Therapy, 14(3): 316 (2006)). Generally, the AAV serotypes have genomic sequences of significant homology at the nucleic acid sequence and amino acid sequence levels, such that different serotypes have an identical set of genetic functions, produce virions which are essentially physically and functionally equivalent, and replicate and assemble by practically identical mechanisms. AAV serotypes 1-6 and 7-9 are defined as “true” serotypes, in that they do not efficiently cross-react with neutralizing sera specific for all other existing and characterized serotypes. In contrast, AAV serotypes 6, 10 (also referred to as Rh10), and 11 are considered “variant” serotypes as they do not adhere to the definition of a “true” serotype. AAV serotype 2 (AAV2) has been used extensively for gene therapy applications due to its lack of pathogenicity, wide range of infectivity, and ability to establish long-term transgene expression (see, e.g., Carter, Hum. Gene Ther., 16:541 (2005); and Wu et al., *supra*). Genome sequences of various AAV serotypes and comparisons thereof are disclosed in, for example, GenBank Accession numbers U89790, J01901, AF043303, and AF085716; Chiorini et al., J. Virol., 71:6823 (1997); Srivastava et al., J. Virol., 45:555 (1983); Chiorini et al., J. Virol., 73:1309 (1999); Rutledge et al., J. Virol., 72:309 (1998); and Wu et al., J. Virol., 74:8635 (2000)).

AAV rep and ITR sequences are particularly conserved across most AAV serotypes. For example, the Rep78 proteins of AAV2, AAV3A, AAV3B, AAV4, and AAV6 are reportedly about 89-93% identical (see Bantel-Schaal et al., J. Virol., 73(2):939 (1999)). It has been reported that AAV serotypes 2, 3A, 3B, and 6 share about 82% total nucleotide sequence identity at the genome level (Bantel-Schaal et al., *supra*). Moreover, the rep sequences and ITRs of many AAV serotypes are known to efficiently cross-complement (e.g., functionally substitute) corresponding sequences from other serotypes during production of AAV particles in mammalian cells.

Generally, the cap proteins, which determine the cellular tropism of the AAV particle, and related cap protein-encoding sequences, are significantly less conserved than Rep genes across different AAV serotypes. In view of the ability Rep and ITR sequences to cross-complement corresponding sequences of other serotypes, the AAV vector can comprise a mixture of serotypes and thereby be a

“chimeric” or “pseudotyped” AAV vector. A chimeric AAV vector typically comprises AAV capsid proteins derived from two or more (e.g., 2, 3, 4, etc.) different AAV serotypes. In contrast, a pseudotyped AAV vector comprises one or more ITRs of one AAV serotype packaged into a capsid of another AAV serotype. Chimeric and pseudotyped AAV vectors are further described in, for example, U.S. Patent No. 6,723,551; Flotte, Mol. Ther., 13(1):1 (2006); Gao et al., J. Virol., 78:6381 (2004); Gao et al., Proc. Natl. Acad. Sci. USA, 99:11854 (2002); De et al., Mol. Ther., 13:67 (2006); and Gao et al., Mol. Ther., 13:77 (2006).

10 In one embodiment, the AAV vector is generated using an AAV that infects humans (e.g., AAV2). Alternatively, the AAV vector is generated using an AAV that infects non-human primates, such as, for example, the great apes (e.g., chimpanzees), Old World monkeys (e.g., macaques), and New World monkeys (e.g., marmosets). In one embodiment, the AAV vector is generated using an AAV that infects a non-human primate pseudotyped with an AAV that infects humans. Examples of such pseudotyped AAV vectors are disclosed in, e.g., Cearley et al., Molecular Therapy, 13:528 (2006). In one embodiment, an AAV vector can be generated which comprises a capsid protein from an AAV that infects rhesus macaques pseudotyped with AAV2 inverted terminal repeats (ITRs). In a particular embodiment, the inventive AAV vector comprises a capsid protein from AAV10 (also referred to as “AAVrh.10”), which infects rhesus macaques pseudotyped with AAV2 ITRs (see, e.g., Watanabe et al., Gene Ther., 17(8):1042 (2010); and Mao et al., Hum. Gene Therapy, 22:1525 (2011)).

25 In addition to the gene to be expressed, the AAV vector may comprise expression control sequences, such as promoters, enhancers, polyadenylation signals, transcription terminators, internal ribosome entry sites (IRES), and the like, that provide for the expression of the nucleic acid sequence in a host cell. Exemplary expression control sequences are known in the art and described in, for example, Goeddel, Gene Expression Technology: Methods in Enzymology, Vol. 185, Academic Press, San Diego, CA. (1990).

A large number of promoters, including constitutive, inducible, and repressible promoters, from a variety of different sources are well known in the art. Representative sources of promoters include for example, virus, mammal, insect, plant, yeast, and bacteria, and suitable promoters from these sources are

readily available, or can be made synthetically, based on sequences publicly available, for example, from depositories such as the ATCC as well as other commercial or individual sources. Promoters can be unidirectional (i.e., initiate transcription in one direction) or bi-directional (i.e., initiate transcription in either a 3' or 5' direction). Non-limiting examples of promoters include, for example, the T7 bacterial expression system, pBAD (araA) bacterial expression system, the cytomegalovirus (CMV) promoter, the SV40 promoter, and the RSV promoter. Inducible promoters include, for example, the Tet system (U.S. Patent Nos. 5,464,758 and 5,814,618), the Ecdysone inducible system (No et al., Proc. Natl. Acad. Sci., 93:3346 (1996)), the T-REXTM system (Invitrogen, Carlsbad, CA), LACSWITCHTM System (Stratagene, San Diego, CA), and the Cre-ERT tamoxifen inducible recombinase system (Indra et al., Nuc. Acid. Res., 27:4324 (1999); Nuc. Acid. Res., 28:e99 (2000); U.S. Patent No. 7,112,715; and Kramer & Fussenegger, Methods Mol. Biol., 308:123 (2005)).

The term "enhancer" as used herein, refers to a DNA sequence that increases transcription of, for example, a nucleic acid sequence to which it is operably linked. Enhancers can be located many kilobases away from the coding region of the nucleic acid sequence and can mediate the binding of regulatory factors, patterns of DNA methylation, or changes in DNA structure. A large number of enhancers from a variety of different sources are well known in the art and are available as or within cloned polynucleotides (from, e.g., depositories such as the ATCC as well as other commercial or individual sources). A number of polynucleotides comprising promoters (such as the commonly-used CMV promoter) also comprise enhancer sequences. Enhancers can be located upstream, within, or downstream of coding sequences. In one embodiment, the nucleic acid sequence is operably linked to a CMV enhancer/chicken beta-actin promoter (also referred to as a "CAG promoter") (see, e.g., Niwa et al., Gene, 108:193 (1991); Daly et al., Proc. Natl. Acad. Sci. U.S.A., 96:2296 (1999); and Sondhi et al., Mol. Ther., 15:481 (2007)).

Typically AAV vectors are produced using well characterized plasmids. For example, human embryonic kidney 293T cells are transfected with one of the transgene specific plasmids and another plasmid containing the adenovirus helper and AAV rep and cap genes (specific to AAVrh.10, 8 or 9 as required). After 72 hours, the cells are harvested and the vector is released from the cells

by five freeze/thaw cycles. Subsequent centrifugation and benzonase treatment removes cellular debris and unencapsidated DNA. Iodixanol gradients and ion exchange columns may be used to further purify each AAV vector. Next, the purified vector is concentrated by a size exclusion centrifuge spin column to the required concentration. Finally, the buffer is exchanged to create the final vector products formulated (for example) in 1x phosphate buffered saline. The viral titers may be measured by TaqMan® real-time PCR and the viral purity may be assessed by SDS-PAGE.

Exemplary Vectors and Exemplary Methods for Targeted Delivery

Striatum is formed by different populations of neurons, which receive and send information to multiple regions in the cerebral cortex and limbic system. Injecting a vector, e.g., an AAV-mediated gene therapy vector, directly into the striatum would result in the expression of gene of interest in all neurons in the targeted area, regardless of their function and connections to other brain structures. This action would result a more nonspecific response in the targeted area, not always beneficial for the evolution of the disease. The present disclosure provides for targeted delivery of a pair of vectors, e.g., targeted to striatal neuronal populations connected to the substantia nigra. For example, dopaminergic neurons in substantia nigra project to the striatum and a RetroAAV.Cre virus delivered in the substantia nigra would migrate via axons and express Cre recombinase in the striatum in a specific neuronal population. Even though the AAV delivered to the striatum would infect all the cells in the area, only the neurons that receive input from the substantia nigra would express the Cre recombinase required for the expression of the AAV-mediated gene of interest. This allows for delivery and manipulation of genes of interest in specific neuronal populations of neuronal circuits in the brain, optionally in a less invasive manner for delivery to the brain than disrupting the skull.

Any method that allows for targeted delivery of the vectors may be employed. In one embodiment any method that allows for targeted delivery of one vector to one site and targeted delivery of the other vector to another site may be employed.

In one embodiment, a pair of rAAV vectors is employed. One of the pairs is a retroAAV that encodes a recombinase, e.g., Cre. In one embodiment, the retroAAV has an AAV2 capsid having at least one or two substitutions that

allow for uptake by nerve terminals in a given brain region, after which it is then transported back to their cell bodies. In one embodiment, the retroAAV is taken up by the neurons of the striatum that project into the substantial nigra.

An exemplary retroAAV capsid sequence is:

5 MAADGYLPDWLEDTLSEGIRQWWKLKPGPPPKPAERHKDDSRGLVLP
 GYKYLGPFNGLDKGEPVNEADAAALEHDKAYDRQLDSGDNPYLKYNH
 ADAEFQERLKEDTSFGGNLGRAVFQAKKRVLEPLGLVEEPVKTAPGKK
 RPVEHSPVEPDSSSGTGKAGQQPARKRLNFGQTGDADSVDPQPLGQPP
 AAPSGLTNTMATGSGAPMADNNEGADGVGNSSGNWHCDSTWMGDR
 10 VITTSTRTWALPTYNNHLYKQISSQSGASNDNHYFGYSTPWGYFDFENRF
 HCHFSPRDWQRLINNNWGFPRKRLNFKLFNIQVKEVTQNDGTTTIANNL
 TSTVQVFTDSEYQLPYVLGSAHQGCLPPFPADVFMVPQYGYLTLNDGSQ
 AVGRSSFYCLEYFPSQMLRTGNNFTFSYTFEDVPFHSSYAHQSGLDRML
 NPLIDQYLYLSRTNTPSGTTTQSRLQFSQAGASDIRDQSRNWLPGPCYR
 15 QQRVSKTSADNNNSEYSWTGATKYHLNGRDSLVPNGPAMASHKDDEE
 KFFPQSGVLIFGKQGSEKTNVDIEKVMITDEEEIRTTNPVATEQYGSVSTN
 LQRGNLADQDYTKTARQAATADVNTQGVLPGMVWQDRDVYLQGPIW
 AKIPHTDGHFHPSPLMGGFGLKHPPPQILIKNTPVPANPSTTFSAAKFASFI
 TQYSTGQVSVEIEWELQKENS KRWNPEIQYTSNYNKSINVDFTVDTNGV
 20 YSEPRPIGTRYLTRNL (SEQ ID NO:1).

The other (non-retro) rAAV of the pair comprises sequences for a gene product of interest that is not expressed due to placement of sites for the recombinase. In one embodiment, the second vector has an AAV1/2 hybrid capsid that is not taken up retrograde. In one embodiment, when the non-retro
 25 rAAV is introduced to the striatum, it only transduces neurons of the striatum and is not taken up into the neurons that project into the striatum from elsewhere.

In one embodiment, at least one or both of the pair of rAAVs is non-invasively administered, e.g., via intravenous injection into the bloodstream,
 30 followed up opening of the blood-brain barrier (BBB) in the relevant target region by MRgFUS so that the virus can get from the bloodstream into that region to transduce those cells. In one example (Figure 1), AAV1/2 with an inactive floxed gene was delivered by intravenous injection into the striatum using MRgFUS targeted to the striatum to focally and transiently open the blood

brain barrier. Under normal circumstances, an active gene would be expressed everywhere in the striatum. Here, however, there would be no expression unless a second vector is present in the same neurons and expresses the recombinase, e.g., cre-recombinase, to flip the construct to the active form to allow for gene expression. At some later point, e.g., later that day, one or more days following MRgFUS or up to several weeks later, after the blood-brain barrier closes again in the striatum, which may happen within 2-24 hours after ultrasound treatment, a second treatment was given, this time with the retroAAV expressing either a recombinase, e.g., Cre, injected into the bloodstream IV and MRgFUS BBB disruption performed in the substantia nigra, so that the retroAAV would only enter the substantia nigra since the BBB was closed in the striatum. In one embodiment, MRgFUS treatments are separated by at least 24 hours to allow the BBB to close in the first area, permitting targeted delivery to the second area. As a result, retroAAV would be taken up into the neurons from the striatum that project into the nigra. When retroAAV-cre is taken up into striatal neurons projecting to the nigra from the striatum, those which already received the inactive floxed gene from the first stratal delivery would then become activated because they would now express Cre, allowing for gene activation. Delivery of retroAAV to the nigra which does not express the appropriate recombinase, e.g., expresses GFP, would fail to activate gene expression. See Figures 4 and 5, confirming that gene expression was targeted exclusively to stratal neurons which project to the substantia nigra.

In one embodiment, invasive delivery may be used to deliver one or more of the vectors, e.g., rAAVs. Invasive deliver includes using surgery to make a hole in the skull and insert a needle into these two targets to directly infuse the respective vectors. That can be done in a single session, rather than two sessions which are used for MRgFUS, since one virus is administered into one region, e.g., via injection, and the other virus into another region.

In one embodiment, direct surgical injection delivers one of the viruses to one target site and MRgFUS is used to deliver the other virus to another target site.

In one embodiment, one region (A) is targeted with the inactive vector and another region (B) is targeted with a retrograde vector expressing the activating gene product (such as Cre). In one embodiment, one region (A) has

the activating gene in a non-retrograde vector and the other region (B) has the inactive gene in a retrograde vector. For example, Cre may be expressed from AAV1/2 delivered to the striatum, followed by delivery of the floxed-DREADD construct in retroAAV to the substantia nigra.

5 In another embodiment, a recombinase encoding vector (a non-retro vector) may be delivered via MRgFUS to the striatum after the desired gene product encoding sequence, e.g., DREADD, is delivered to the striatum, e.g., via MRgFUS. There is no disadvantage to disrupting the BBB in the same location multiple times.

10 In one embodiment, the pair of vectors may be employed to prevent, inhibit or treat a neurological disease or disorder via neurons. In one embodiment, dopaminergic projections from the ventral tegmental area (VTA) to the nucleus accumbent (NAc) are targeted or VTA projections to medial prefrontal cortex (mPFC) are targeted. Activating VTA-NAc promotes
15 depression whereas activating VTA-mPFC blocks depression even though they both come from the same VTA region. Addiction may be similarly influenced by manipulating these circuits. Speech or swallowing dysfunction may be improved by targeting a specific projection from the periaqueductal gray (PAG) to the nucleus ambiguus (NAmb). Other disorders that may be treated using
20 specific circuits in the brain include but are not limited to obesity and feeding behavior, anxiety, neuroendocrine problems, and the like.

Pharmaceutical Compositions and Delivery

 The disclosure provides one or more compositions comprising, consisting essentially of, or consisting of the above-described gene transfer
25 vectors and a pharmaceutically acceptable (e.g., physiologically acceptable) carrier. When the composition consists essentially of a gene transfer vector and a pharmaceutically acceptable carrier, additional components can be included that do not materially affect the composition (e.g., adjuvants, buffers, stabilizers, anti-inflammatory agents, solubilizers, preservatives, etc.). When the
30 composition consists of the gene transfer vector and the pharmaceutically acceptable carrier, the composition does not comprise any additional components. Any suitable carrier can be used within the context of the disclosure, and such carriers are well known in the art. The choice of carrier will be determined, in part, by the particular site to which the composition may be

administered and the particular method used to administer the composition. The composition optionally can be sterile with the exception of the gene transfer vector described herein. The composition can be frozen or lyophilized for storage and reconstituted in a suitable sterile carrier prior to use. The compositions can be generated in accordance with conventional techniques described in, e.g., *Remington: The Science and Practice of Pharmacy, 21st Edition*, Lippincott Williams & Wilkins, Philadelphia, PA (2001).

Suitable formulations for the composition include aqueous and non-aqueous solutions, isotonic sterile solutions, which can contain anti-oxidants, buffers, and bacteriostats, and aqueous and non-aqueous sterile suspensions that can include suspending agents, solubilizers, thickening agents, stabilizers, and preservatives. The formulations can be presented in unit-dose or multi-dose sealed containers, such as ampules and vials, and can be stored in a freeze-dried (lyophilized) condition requiring only the addition of the sterile liquid carrier, for example, water, immediately prior to use. Extemporaneous solutions and suspensions can be prepared from sterile powders, granules, and tablets of the kind previously described. In one embodiment, the carrier is a buffered saline solution. In one embodiment, the inventive gene transfer vector is administered in a composition formulated to protect the gene transfer vector from damage prior to administration. For example, the composition can be formulated to reduce loss of the gene transfer vector on devices used to prepare, store, or administer the gene transfer vector, such as glassware, syringes, or needles. The composition can be formulated to decrease the light sensitivity and/or temperature sensitivity of the gene transfer vector. To this end, the composition may comprise a pharmaceutically acceptable liquid carrier, such as, for example, those described above, and a stabilizing agent selected from the group consisting of polysorbate 80, L-arginine, polyvinylpyrrolidone, trehalose, and combinations thereof. Use of such a composition will extend the shelf life of the gene transfer vector, facilitate administration, and increase the efficiency of the inventive method. Formulations for gene transfer vector -containing compositions are further described in, for example, Wright et al., *Curr. Opin. Drug Discov. Devel.*, 6(2): 174-178 (2003) and Wright et al., *Molecular Therapy*, 12: 171-178 (2005))

The composition also can be formulated to enhance transduction efficiency. In addition, one of ordinary skill in the art will appreciate that the gene transfer vector can be present in a composition with other therapeutic or biologically-active agents. For example, factors that control inflammation, such as ibuprofen or steroids, can be part of the composition to reduce swelling and inflammation associated with *in vivo* administration of the gene transfer vector. Immune system stimulators or adjuvants, e.g., interleukins, lipopolysaccharide, and double-stranded RNA, can be administered to enhance or modify the immune response. Antibiotics, i.e., microbicides and fungicides, can be present to treat existing infection and/or reduce the risk of future infection, such as infection associated with gene transfer procedures.

Injectable depot forms are made by forming microencapsule matrices of the subject compounds in biodegradable polymers such as polylactide-polyglycolide. Depending on the ratio of drug to polymer, and the nature of the particular polymer employed, the rate of drug release can be controlled. Examples of other biodegradable polymers include poly(orthoesters) and poly(anhydrides). Depot injectable formulations are also prepared by entrapping the drug in liposomes or microemulsions which are compatible with body tissue.

In certain embodiments, a formulation of the present disclosure comprises a biocompatible polymer selected from the group consisting of polyamides, polycarbonates, polyalkylenes, polymers of acrylic and methacrylic esters, polyvinyl polymers, polyglycolides, polysiloxanes, polyurethanes and copolymers thereof, celluloses, polypropylene, polyethylenes, polystyrene, polymers of lactic acid and glycolic acid, polyanhydrides, poly(ortho)esters, poly(butic acid), poly(valeric acid), poly(lactide-co-caprolactone), polysaccharides, proteins, polyhyaluronic acids, polycyanoacrylates, and blends, mixtures, or copolymers thereof.

The composition can be administered in or on a device that allows controlled or sustained release, such as a sponge, biocompatible meshwork, mechanical reservoir, or mechanical implant. Implants (see, e.g., U.S. Patent No. 5,443,505), devices (see, e.g., U.S. Patent No. 4,863,457), such as an implantable device, e.g., a mechanical reservoir or an implant or a device comprised of a polymeric composition, are particularly useful for administration of the inventive gene transfer vector. The composition also can be administered

in the form of sustained-release formulations (see, e.g., U.S. Patent No. 5,378,475) comprising, for example, gel foam, hyaluronic acid, gelatin, chondroitin sulfate, a polyphosphoester, such as bis-2-hydroxyethyl-terephthalate (BHET), and/or a polylactic-glycolic acid.

5 Delivery of the compositions comprising the gene transfer vectors may be intracerebral (including but not limited to intraparenchymal, intraventricular, or intracisternal), intrathecal (including but not limited to lumbar or cisterna magna), or systemic, including but not limited to intravenous, or any combination thereof, using devices known in the art. Delivery may also be via
10 surgical implantation of an implanted device.

 The dose of the gene transfer vector in the composition administered to the mammal will depend on a number of factors, including the size (mass) of the mammal, the extent of any side-effects, the particular route of administration, and the like. In one embodiment, the inventive method comprises administering
15 a “therapeutically effective amount” of the composition comprising the inventive gene transfer vector described herein. A “therapeutically effective amount” refers to an amount effective, at dosages and for periods of time necessary, to achieve a desired therapeutic result. The therapeutically effective amount may vary according to factors such as pathology, age, sex, and weight of the
20 individual, and the ability of the gene transfer vector to elicit a desired response in the individual. The dose of gene transfer vector in the composition required to achieve a particular therapeutic effect typically is administered in units of vector genome copies per cell (gc/cell) or vector genome copies/per kilogram of body weight (gc/kg). One of ordinary skill in the art can readily determine an
25 appropriate gene transfer vector dose range to treat a patient having a particular disease or disorder, based on these and other factors that are well known in the art. The therapeutically effective amount may be between 1×10^{10} genome copies to 1×10^{13} genome copies.

 In one embodiment, the vector is an adenovirus, adeno-associated virus
30 (AAV), retrovirus or lentivirus vector. In one embodiment, the AAV vector is pseudotyped. In one embodiment, the AAV vector is pseudotyped with AAVrh.10, AAV8, AAV9, AAV5, AAVhu.37, AAVhu.20, AAVhu.43, AAVhu.8, AAVhu.2, or AAV7 capsid. In one embodiment, the AAV vector is pseudotyped with AAVrh.10, AAV8, or AAV5. In one embodiment, the AAV

vector is AAV2, AAV5, AAV7, AAV8, AAV9 or AAVrh.10. Further provided is a pharmaceutical composition comprising an amount of the gene therapy vector described above. A dose of the viral vector may be about 1×10^{11} to about 1×10^{16} genome copies, about 1×10^{12} to about 1×10^{15} genome copies
5 about 1×10^{11} to about 1×10^{13} genome copies, or about 1×10^{13} to about 1×10^{15} genome copies.

In one embodiment, the composition is administered once to the mammal. It is believed that a single administration of the composition will result in persistent expression in the mammal with minimal side effects.

10 However, in certain cases, it may be appropriate to administer the composition multiple times during a therapeutic period to ensure sufficient exposure of cells to the composition. For example, the composition may be administered to the mammal two or more times (e.g., 2, 3, 4, 5, 6, 6, 8, 9, or 10 or more times) during a therapeutic period.

15 Exemplary Embodiments

In one embodiment, the disclosure provides compositions and methods for non-invasive targeting of specific neuronal circuits and for non-invasive regulation of the activity of specific neuronal circuits. In one embodiment, at least two gene therapy vectors are employed. In one embodiment, one
20 retrograde viral vector is delivered to an organ, brain or spinal cord region of a mammal with a population of axons that come from afferent neurons. In one embodiment, the viral vector contains an inactive form of a gene which, when activated, can modulate neuronal function. A second vector is delivered to one brain region of the mammal that sends a limited number of projections to the
25 first target, e.g., organ, brain or spinal cord region with a population of axons that come from afferent neuron.

In one embodiment, one vector expresses a gene encoding a protein that activates the gene or gene product in the other vector. In one example, a retrograde vector expresses Cre recombinase within the nervous system region
30 that projects to the target, while the other vector, e.g., which contains a conditionally inactivated gene flanked by lox sites which recombine to activate the gene in the presence of Cre, thereby allowing for expression of the gene in the target site. Another example uses a similar approach with the FLP recombinase system.

In one embodiment, the second viral vector expresses a gene encoding a protein that activates the gene or gene product in the first viral vector. In one example, the second vector expresses Cre recombinase, e.g., it is injected or indirectly targeted to the target region within the nervous system, while the retrograde vector, e.g., which is injected or indirectly targeted a region that projects to the target region, contains an inactive gene flanked by lox sites which recombine to activate the gene in the presence of Cre. Another example uses a similar approach with the FLP recombinase system.

In one embodiment, one vector expresses a gene encoding a protein that activates the gene or gene product in the other vector. In one example, one vector expresses Cre recombinase within the nervous system region that projects to the first target, while the retrograde vector, e.g., which is injected into the first region, contains an inactive gene flanked by lox sites which recombine to activate the gene in the presence of Cre. Another example uses a similar approach with the FLP recombinase system.

In yet another example, one vector, e.g., the first vector which is optionally a retrograde vector, contains a therapeutic gene controlled by the Tet “On” promoter, while the second vector is injected, e.g., systemically or to a targeted region, into, for example, a second brain region, expresses the rTTA transactivator that drives expression from the Tet “On” promoter in the presence of tetracycline. These may be delivered through invasive injection directly into the target organ and/or central nervous system regions, allowing subsequent non-invasive control of neuronal function through expression of genes such as ion channels, G proteins or transcription factors, which respond to exogenous drugs or stimuli to regulate neuronal function.

In one embodiment, the disclosure also provides for a non-invasive method to target specific circuits in the brain. This method utilizes focal disruption of the blood-brain barrier (BBB) with focused ultrasound, which can transiently open the BBB in regions targeted by the ultrasound when microbubbles, e.g., Optison (GE Healthcare) or Definity (Lantheus Medical Imaging) or another cavitating agent is given intravenously. In one example, a vector, which is optionally a retrograde vector such as a retrograde AAV vector, containing an inactive form of a Designer Receptor Exclusively Activated by Designer Drugs (DREADD) hD3q which is flanked by lox sites, is delivered

through focused ultrasound to the substantia nigra. The vector delivers the gene to local substantia nigra neurons and to neurons which project into the substantia nigra, one of which is the putamen. In one embodiment, about 48 hours later, after the BBB has closed and systemic AAV can no longer efficiently enter the

5 brain, a second AAV serotype, e.g., serotype 1, vector is delivered to the putamen with a second session of focused ultrasound. In one example, this vector contains the gene for Cre under the control of a cytomegalovirus (CMV) promoter with a chicken beta-actin (CBA) enhancer, and the Cre open reading frame is flanked by lox sites. When the Cre is expressed in the putaminal

10 neurons, it then recombines the DREADD, which is only contained with putaminal neurons projecting to the substantia nigra, thereby activating the DREADD only in these neurons. The same Cre enzyme also recombines the Cre gene to delete and separate the Cre open reading frame from the promoter, thereby stopping further Cre expression, which is no longer needed. The result

15 is expression of a DREADD only within striatonigral neurons (the specific putamen-substantia nigra neurons), and following administration of the drug clozapine-N-oxide (CNO), the DREADD is activated, thereby activating specifically the nigrostriatal neurons to improve symptoms of, for example, Parkinson's disease. In other examples, the gene delivered to conditionally

20 activate the striatonigral neurons includes ion channels which respond to chemicals (chemogenetics), ultrasound (sonogenetics), and/or magnetic fields (magnetogenetics). In one embodiment, the vectors are used to conditionally overexpress genes that either prevent or delay the onset of a disease, or silence genes that are associated with an increased risk of developing a disease. For

25 example, overexpression of ApoE2 has protective effects in regard to Alzheimer's disease (AD) while LDLR overexpression decreases the amyloid deposition delaying development of AD pathology. In addition, genes associated with an increased risk for a disease can be silenced: for example, ApoE4 or RAB10 (Alzheimer's disease), and PINK1, PARK1/4, or LRRK2 (Parkinson's

30 disease).

In one example, a retrograde vector such as a retrograde AAV vector, containing Cre under the control of a cytomegalovirus (CMV) promoter with a chicken beta-actin (CBA) enhancer that is delivered through focused ultrasound to the substantia nigra is employed with a non-retrograde vector having

an inactive form of a gene such as a Designer Receptor Exclusively Activated by Designer Drugs (DREADD) hD3q which is flanked by lox sites, that is delivered through focused ultrasound to the striatum. The vectors deliver the genes to local neurons in distinct regions, e.g., substantia nigra neurons and to neurons

5 which project into the substantia nigra. In one embodiment, after one of the vectors is delivered to one of the sites using MRfUS, e.g., about 48 hours later, after the BBB has closed and systemic AAV can no longer efficiently enter the brain, a second AAV vector, optionally of a different serotype, is delivered to the second site with a second session of focused ultrasound. In one example, a

10 retrograde vector contains the gene for Cre under the control of a cytomegalovirus (CMV) promoter with a chicken beta-actin (CBA) enhancer. When the Cre is delivered to the same cells as those with the conditionally inactivated gene that is flanked by lox sites, it recombines the gene, e.g., DREADD, thereby activating it only in the neurons where the vector having the

15 gene was delivered. The result is expression of a DREADD, e.g., only within striatonigral neurons, and following administration of the drug clozapine-N-oxide (CNO), the DREADD is activated, thereby activating the neurons with DREADD to improve symptoms of, for example, Parkinson's disease. In other examples, the conditionally inactivated gene includes ion channels which

20 respond to chemicals (chemogenetics), ultrasound (sonogenetics), and/or magnetic fields (magnetogenetics).

Diseases or disorders that may benefit from use of the vectors described herein include but are not limited to Acquired Epileptiform Aphasia, Acute Disseminated Encephalomyelitis, Adrenoleukodystrophy, Agenesis of the corpus

25 callosum, Agnosia, Aicardi syndrome, Alexander disease, Alpers' disease, Alternating hemiplegia, Alzheimer's disease, Amyotrophic lateral sclerosis (see Motor Neuron Disease), Anencephaly, Angelman syndrome, Angiomatosis, Anoxia, Aphasia, Apraxia, Arachnoid cysts, Arachnoiditis, Arnold-Chiari malformation, Arteriovenous malformation, Asperger's syndrome, Ataxia

30 Telangiectasia, Attention Deficit Hyperactivity Disorder, Autism, Auditory processing disorder, Autonomic Dysfunction, , Back Pain, Batten disease, Behcet's disease, Bell's palsy, Benign Essential Blepharospasm, Benign Focal Amyotrophy, Benign Intracranial Hypertension, Bilateral frontoparietal polymicrogyria, Binswanger's disease, Blepharospasm, Bloch-Sulzberger

syndrome, Brachial plexus injury, Brain abscess, Brain damage, Brain injury,
 Brain tumor, Brown-Sequard syndrome, Canavan disease, Carpal tunnel
 syndrome (CTS), Causalgia, Central pain syndrome, Central pontine
 myelinolysis, Centronuclear myopathy, Cephalic disorder, Cerebral aneurysm,
 5 Cerebral arteriosclerosis, Cerebral atrophy, Cerebral gigantism, Cerebral palsy,
 Charcot-Marie-Tooth disease, Chiari malformation, Chorea, Chronic
 inflammatory demyelinating polyneuropathy (CIDP), Chronic pain, Chronic
 regional pain syndrome, Coffin Lowry syndrome, Coma, including Persistent
 Vegetative State, Congenital facial diplegia, Corticobasal degeneration, Cranial
 10 arteritis, Craniosynostosis, Creutzfeldt-Jakob disease, Cumulative trauma
 disorders, Cushing's syndrome, Cytomegalic inclusion body disease (CIBD),
 Cytomegalovirus Infection, , Dandy-Walker syndrome, Dawson disease, De
 Morsier's syndrome, Dejerine-Klumpke palsy, Dejerine-Sottas disease, Delayed
 sleep phase syndrome, Dementia, Dermatomyositis, Neurological Dyspraxia,
 15 Diabetic neuropathy, Diffuse sclerosis, Dysautonomia, Dyscalculia, Dysgraphia,
 Dyslexia, Dystonia, , Early infantile epileptic encephalopathy, Empty sella
 syndrome, Encephalitis, Encephalocele, Encephalotrigeminal angiomatosis,
 Encopresis, Epilepsy, Erb's palsy, Erythromelalgia, Essential tremor (ET),
 Fabry's disease, Fahr's syndrome, Fainting, Familial spastic paralysis, Febrile
 20 seizures, Fisher syndrome, Friedreich's ataxia, FART Syndrome, Gaucher's
 disease, Gerstmann's syndrome, Giant cell arteritis, Giant cell inclusion disease,
 Globoid cell Leukodystrophy, Gray matter heterotopia, Guillain-Barre
 syndrome, HTLV-1 associated myelopathy, Hallervorden-Spatz disease, Head
 injury, Headache, Hemifacial Spasm, Hereditary Spastic Paraplegia,
 25 Heredopathia atactica polyneuritiformis, Herpes zoster oticus, Herpes zoster,
 Hirayama syndrome, Holoprosencephaly, Huntington's disease,
 Hydranencephaly, Hydrocephalus, Hypercortisolism, Hypoxia, Immune-
 Mediated encephalomyelitis, Inclusion body myositis, Incontinentia pigmenti,
 Infantile phytanic acid storage disease, Infantile Refsum disease, Infantile
 30 spasms, Inflammatory myopathy, Intracranial cyst, Intracranial hypertension,
 Joubert syndrome, Kearns-Sayre syndrome, Kennedy disease, Kinsbourne
 syndrome, Klippel Feil syndrome, Krabbe disease, Kugelberg-Welander disease,
 Kuru, Lafora disease, Lambert-Eaton myasthenic syndrome, Landau-Kleffner
 syndrome, Lateral medullary (Wallenberg) syndrome, Learning disabilities,

- Leigh's disease, Lennox-Gastaut syndrome, Lesch-Nyhan syndrome, Leukodystrophy, Lewy body dementia, Lissencephaly, Locked-In syndrome, Lou Gehrig's disease, Lumbar disc disease, Lyme disease - Neurological Sequelae, Machado-Joseph disease (Spinocerebellar ataxia type 3),
- 5 Macrencephaly, Maple Syrup Urine Disease, Megalencephaly, Melkersson-Rosenthal syndrome, Menieres disease, Meningitis, Menkes disease, Metachromatic leukodystrophy, Microcephaly, Migraine, Miller Fisher syndrome, Mini-Stroke, Mitochondrial Myopathies, Mobius syndrome, Monomelic amyotrophy, Motor Neuron Disease, Motor skills disorder,
- 10 Moyamoya disease, Mucopolysaccharidoses (including the subset referred to as Hurler Syndrome, Hurler-Scheie syndrome, Scheie syndrome, Hunter syndrome, Sanfilippo syndromes A-D, Morquio syndromes A and B, Maroteaux-Lamy syndrome, Sly syndrome, and Natowicz syndrome), Multi-Infarct Dementia, Multifocal motor neuropathy, Multiple sclerosis, Multiple system atrophy with
- 15 postural hypotension, Muscular dystrophy, Myalgic encephalomyelitis, Myasthenia gravis, Myelinoclastic diffuse sclerosis, Myoclonic Encephalopathy of infants, Myoclonus, Myopathy, Myotubular myopathy, Myotonia congenita, Narcolepsy, Neurofibromatosis, Neuroleptic malignant syndrome, Neurological manifestations of AIDS, Neurological sequelae of lupus,
- 20 Neuromyotonia, Neuronal ceroid lipofuscinosis, Neuronal migration disorders, Niemann-Pick disease, Non 24-hour sleep-wake syndrome, Nonverbal learning disorder, , O'Sullivan-McLeod syndrome, Occipital Neuralgia, Occult Spinal Dysraphism Sequence, Ohtahara syndrome, Olivopontocerebellar atrophy, Opsoclonus myoclonus syndrome, Optic neuritis, Orthostatic Hypotension,
- 25 Overuse syndrome, Palinopsia, Paresthesia, Parkinson's disease, Paramyotonia Congenita, Paraneoplastic diseases, Paroxysmal attacks, Parry-Romberg syndrome (also known as Rombergs Syndrome), Pelizaeus-Merzbacher disease, Periodic Paralysis, Peripheral neuropathy, Persistent Vegetative State, Pervasive NDs, Photic sneeze reflex, Phytanic Acid Storage disease, Pick's disease,
- 30 Pinched Nerve, Pituitary Tumors, PMG, Polio, Polymicrogyria, Polymyositis, Porencephaly, Post-Polio syndrome, Postherpetic Neuralgia (PHN), Postinfectious Encephalomyelitis, Postural Hypotension, Prader-Willi syndrome, Primary Lateral Sclerosis, Prion diseases, Progressive Hemifacial Atrophy also known as Rombergs_Syndrome, Progressive multifocal leukoencephalopathy,

Progressive Sclerosing Poliodystrophy, Progressive Supranuclear Palsy, Pseudotumor cerebri, Ramsay-Hunt syndrome (Type I and Type II), Rasmussen's encephalitis, Reflex sympathetic dystrophy syndrome, Refsum disease, Repetitive motion disorders, Repetitive stress injury, Restless legs syndrome, Retrovirus-associated myelopathy, Rett syndrome, Reye's syndrome, Rombergs_Syndrome, Rabies, Saint Vitus dance, Sandhoff disease, Schytsophrenia, Schilder's disease, Schizencephaly, Sensory Integration Dysfunction, Septo-optic dysplasia, Shaken baby syndrome, Shingles, Shy-Drager syndrome, Sjogren's syndrome, Sleep apnea, Sleeping sickness, Sniatiation, Sotos syndrome, Spasticity, Spina bifida, Spinal cord injury, Spinal cord tumors, Spinal muscular atrophy, Spinal stenosis, Steele-Richardson-Olszewski syndrome, see Progressive Supranuclear Palsy, Spinocerebellar ataxia, Stiff-person syndrome, Stroke, Sturge-Weber syndrome, Subacute sclerosing panencephalitis, Subcortical arteriosclerotic encephalopathy, Superficial siderosis, Sydenham's chorea, Syncope, Synesthesia, Syringomyelia, Tardive dyskinesia, Tay-Sachs disease, Temporal arteritis, Tethered spinal cord syndrome, Thomsen disease, Thoracic outlet syndrome, Tic Douloureux, Todd's paralysis, Tourette syndrome, Transient ischemic attack, Transmissible spongiform encephalopathies, Transverse myelitis, Traumatic brain injury, Tremor, Trigeminal neuralgia, Tropical spastic paraparesis, Trypanosomiasis, Tuberous sclerosis, Vasculitis including temporal arteritis, Von Hippel-Lindau disease (VHL), Viliuisk Encephalomyelitis (VE), Wallenberg's syndrome, Werdnig-Hoffman disease, West syndrome, Whiplash, Williams syndrome, Wilson's disease, X-Linked Spinal and Bulbar Muscular Atrophy, or Zellweger syndrome.

In one embodiment, the method for targeting a specific population of mammalian neurons for activatable expression of a gene product includes administering an effective amount of a composition comprising a viral vector with a conditionally activatable gene or gene product, which viral vector is capable of retrograde uptake into neurons to a structure in the mammal to be modulated which contains axons from regulatory neurons, and a composition comprising a second viral vector expressing a gene, the product of which can activate the gene in the first viral vector, which second vector is directly or

indirectly targeted to a different region containing a subset of the cell bodies that send the axons to the structure to be modulated.

In one embodiment, the method for targeting a specific population of mammalian neurons for activatable expression of a gene product includes
5 administering an effective amount of a composition comprising a first viral vector with a conditionally activatable gene or gene product, to a structure in the central nervous system of the mammal to be modulated, and a composition comprising a second viral vector expressing a gene, the product of which can activate the gene in the first viral vector, to a different region in the central
10 nervous system that sends axons to the structure to be modulated, which second viral vector is capable of retrograde uptake into neurons.

In one embodiment, the method for targeting a specific population of mammalian neurons for activatable expression of a gene product includes administering an effective amount of a composition comprising a first viral
15 vector with a conditionally activatable gene or gene product, to a structure in the central nervous system of the mammal to be modulated, and a composition comprising a second viral vector expressing a gene, the product of which can activate the gene in the first viral vector, wherein one of the vectors is capable of retrograde uptake into neurons.

In one embodiment, the method for targeting a specific population of mammalian neurons for activatable expression of a gene product includes administering an effective amount of a composition comprising a first viral
20 vector with a conditionally activatable gene or gene product, to a structure in the central nervous system of the mammal to be modulated, and a composition comprising a second viral vector expressing a gene, the product of which can activate the gene in the first viral vector, to the structure.
25

In one embodiment, the first vector is systemically administered. In one embodiment, the first vector is locally administered. In one embodiment, the second vector is systemically administered. In one embodiment, the second
30 vector is locally administered. In one embodiment, focused ultrasound is employed for targeted delivery of at least one vector. In one embodiment, focused ultrasound is employed for sequential targeted delivery of the first and second vector, e.g., to the same or to different sites. In one embodiment, focused ultrasound is employed for concurrent targeted delivery of the first and second

vector, e.g., to the same region. In one embodiment, both vectors are injected directly into the brain into the same region, at the same time (e.g., day) or sequentially, e.g., on the same or different days. In one embodiment, both vectors are injected directly into the brain at different sites at the same time or sequentially, e.g., on the same or different days. In one embodiment, both vectors are targeted to the brain, e.g., after systemic administration and focused ultrasound, into the same region, e.g., sequential administration on different days. In one embodiment, both vectors are targeted to the brain, e.g., after systemic administration and focused ultrasound, to different sites, e.g., sequential administration on different days. In one embodiment, invasive delivery, e.g., via opening up the skull, is employed for one or both vectors. In one embodiment, invasive delivery is employed for one vector and non-invasive delivery, e.g., systemic injection and focused ultrasound, is employed for the other vector to the same or a different site than the vector that is invasively delivered. In one embodiment, expression of the conditionally activatable gene is in the brain. In one embodiment, expression of the conditionally activatable gene is in a peripheral organ. In one embodiment, the viral vector comprises an adeno-associated virus, adenovirus, canine adenovirus, herpes simplex virus, or lentivirus vector. In one embodiment, the viral vector comprises a retrograde form of adeno-associated virus, lentivirus or canine adenovirus. In one embodiment, the first viral vector contains recognition sites capable of recombination and activation of the gene in the first vector and the second vector encodes a recombinase that is specific for the recognition sites. In one embodiment, the recombinase comprises Cre or Flp. In one embodiment, the gene product is activatable by an exogenous agent. In one embodiment, the method includes administering the exogenous agent to the mammal. In one embodiment, the gene product prevents or inhibits one or more symptoms of a neurological disease or disorder. In one embodiment, the recombinase is conditionally activated by an exogenous chemical or stimulus. In one embodiment, the gene in the first viral vector does not express a functional protein autonomously, and the second vector expresses a protein that is capable of activating gene expression or protein function from the gene in the viral vector. In one embodiment, the second vector expresses a regulatable or

autonomously active transcription factor capable of transactivating expression of the gene product encoded from the viral vector. In one embodiment, at least one of the viral vectors is delivered via direct infusion into the structure. In one embodiment, at least one of the viral vectors is delivered through focal

5 disruption of the blood-brain barrier using focused ultrasound. In one embodiment, m one of the viral vectors is targeted to one brain region in a single treatment, followed by targeting of the other vector to a second brain region in a second treatment. In one embodiment, the two treatments are separated by at least 24 hours. In one embodiment, the mammal is a human. In one embodiment,

10 the mammal is a non-human primate. In one embodiment, the mammal is rodent, swine, caprine, ovine, bovine, equine, canine or feline.

All publications, patents and patent applications are incorporated herein by reference. While in the foregoing specification, this invention has been described in relation to certain embodiments thereof, and many details have been

15 set forth for purposes of illustration, it will be apparent to those skilled in the art that the invention is susceptible to additional embodiments and that certain of the details herein may be varied considerably without departing from the basic principles of the invention.