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

SUMMARY

A method for targeting a specific population of neurons in a mammal, e.g., a human, is provided. In one example, 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 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 example, the method comprises delivery of a viral vector with a conditionally activatable gene or gene 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 example, the method comprises delivery of a first viral vector with a conditionally activatable gene or gene product, which first vector is capable of 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 example, 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 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 example, the structure to be modulated is a peripheral organ or a brain region. In one example, the viral vector is an adeno-associated virus, adenovirus, canine adenovirus, herpes simplex virus, or 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 example, 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 example, the recombinase is Cre or Flp. In one example, a recombinase enzyme is conditionally activated by an exogenous chemical or stimulus.

In one aspect, the present disclosure refers to use of i) an amount of a first composition comprising a first viral vector comprising a conditionally activatable gene or gene product, and ii) an amount of a second composition comprising a second viral vector comprising an open reading frame for expressing a gene, the product of which activates the gene in the first viral vector, in the manufacture of a medicament for targeting a specific population of mammalian neurons for activatable expression of a gene product in the central nervous system of a mammal, wherein the amount of the second composition is effective to allow for expression of the gene or gene product in the first viral vector in the specific population of neurons in the mammal relative to a mammal not administered the first and second viral vector.

In one aspect, the present disclosure refers to use of an amount of a composition comprising a viral vector comprising a conditionally activatable gene or gene product, in the manufacture of a medicament for targeting a specific population of mammalian neurons for activatable expression of a gene product in the central nervous system of a mammal, wherein the mammal comprises a vector comprising an open reading frame for expressing a gene, the product of which activates the gene in the viral vector, wherein the amount is effective to allow for expression of the gene or gene product in the viral vector in the specific population of neurons in the mammal.

In one aspect, the present disclosure refers to use of an amount of a composition comprising a viral vector comprising an open reading frame for a gene, the product of which activates a conditionally activatable gene or gene product, in the manufacture of a medicament for targeting a specific population of mammalian neurons for activatable expression of a gene product in the central nervous system of a mammal, wherein the mammal comprises a vector comprising the conditionally activatable gene or gene product, wherein the amount is effective to allow for expression of the gene or gene product in the viral vector in the specific population of neurons in the mammal.

In one aspect, the present disclosure refers to use of a first viral vector comprising a conditionally activatable gene or gene product and a second viral vector comprising an open reading frame for expressing a gene, the product of which activates the gene in the viral vector, in the manufacture of a medicament for preventing, inhibiting or treating one or more symptoms of a neurological disease.

In one aspect, the present disclosure refers to use of a first viral vector in the manufacture of a first medicament and use of a second viral vector in the manufacture of a second medicament, wherein the first and second medicaments are to be used in combination to target a specific population of mammalian neurons for activatable expression of a gene product in the central nervous system of a mammal, wherein the first viral vector comprises a conditionally activatable gene or gene product, and wherein the second viral vector comprises an open reading frame for expressing a gene product which activates the gene in or gene product of the first viral vector, in the manufacture of a medicament for targeting a specific population of mammalian neurons for activatable expression of a gene product in the central nervous system of a mammal.

In one example, 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 example, the second vector expresses regulatable or autonomously active transcription factors capable of transactivating expression of the gene, e.g., encoding a therapeutic gene product, from the first vector.

In one example, the viral vector is delivered via direct infusion into the organ or brain region. In one example, the viral vector is delivered through focal disruption of the blood-brain barrier using focused ultrasound. In one example, 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 example, the two treatments are separated by at least 24, 36 or 72 hours or more. In one example, the two treatments are sequential, e.g., occur on the same day. In one example, the two treatments are conducted simultaneously. In one example, 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, wherein optionally one of the vectors is capable of retrograde uptake in neurons. In one example, the vectors are both viral vectors, e.g., AAV vectors that may differ in serotype. In one example, the first vector encodes an ion channel protein. In one example, the first vector encodes a G coupled protein receptor ion channel protein. In one example, the first vector encodes a transcription factor.

In one example, 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 example, one of the vectors is a recombinant viral vector, e.g., rAAV vector. In one example, both of the vectors are recombinant viral vectors. In one example, when both of the vectors are recombinant viral vectors, one is a retrograde viral vector.

In one example, non-invasive delivery of two vectors is employed. In one example, 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 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 example, 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.

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

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

BRIEF DESCRIPTION OF FIGURES

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

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.