VECTORS AND USE OF SAME IN GENETIC TREATMENT FOR NMDA RECEPTOR-RELATED ENCEPHALOPATHIES

REFERENCE TO AN ELECTRONIC SEQUENCE LISTING

[0001] The contents of the electronic sequence listing (TECH-P-0306-USP.xml; size: 14,336 bytes; and date of creation: March 16, 2025) is herein incorporated by reference in its entirety.

FIELD OF INVENTION

[0002] The present disclosure relates *inter alia* to expression vectors, as well as methods of using same, such as for genetic treatments of neurological disorders.

BACKGROUND

[0003] N-methyl-D-aspartate (NMDA) receptors are ionotropic glutamate receptors that play a crucial role in synaptic plasticity, learning, and memory in the central nervous system. These receptors are heterotetrameric complexes composed of different subunit combinations, including GluN1, GluN2A-D, and GluN3A-B subunits. The specific subunit composition determines the receptor's functional properties and physiological roles.

[0004] In recent years, there has been growing recognition of the involvement of NMDA receptor dysfunction in various neurological and psychiatric disorders. Mutations in genes encoding NMDA receptor subunits have been associated with a spectrum of neurodevelopmental disorders, collectively referred to as NMDA receptor-related encephalopathies. These conditions can manifest with a range of symptoms, including intellectual disability, epilepsy, autism spectrum disorders, and movement abnormalities.

[0005] The severity and specific clinical presentation of NMDA receptor-related encephalopathies can vary depending on the affected subunit and the nature of the mutation. Some mutations may lead to loss- or gain-of-function, while others may result in altered receptor kinetics or trafficking, etc. Understanding the molecular mechanisms

underlying these disorders is an active area of research, as it may provide insights into potential therapeutic strategies.

[0006] Current treatment options for NMDA receptor-related encephalopathies are limited and primarily focused on symptom management. Antiepileptic drugs are often used to control seizures, while other supportive therapies may be employed to address specific symptoms. However, these approaches do not address the underlying genetic cause of the disorder.

[0007] Gene therapy has emerged as a promising approach for treating genetic disorders, including those affecting the central nervous system. Adeno-associated virus (AAV) vectors have gained attention as potential tools for gene delivery due to their ability to transduce neurons efficiently and their relatively low immunogenicity. However, challenges remain in developing effective gene therapy strategies for NMDA receptor-related encephalopathies, including the need for efficient delivery across the blood-brain barrier and —importantly—achieving appropriate expression levels of the therapeutic gene.

[0008] As research in this field progresses, there is a need for improved methods to deliver functional NMDA receptor subunit genes to affected neurons in the central nervous system. Such approaches could potentially restore proper receptor function and alleviate the symptoms associated with NMDA receptor-related encephalopathies. Additionally, the development of targeted gene therapy strategies may offer new possibilities for personalized treatment based on an individual's specific genetic mutation.

SUMMARY

[0009] This summary is provided to introduce a selection of concepts in a simplified form that are further described below in the detailed description. This summary is not intended to identify key features or essential features of the claimed subject matter, nor is it intended to be used as an aid in determining the scope of the claimed subject matter.

[0010] According to the first aspect, there is provided a recombinant adeno-associated virus (AAV) vector comprising: (a) a nucleic acid sequence encoding an N-methyl-D-aspartate (NMDA) receptor subunit; and (b) a promoter having a length ranging between 50 and 500 bp, wherein the nucleic acid sequence and the promoter are operably linked.

[0011] According to another aspect, there is provided a pharmaceutical composition comprising a therapeutically effective amount of the recombinant AAV vector of the invention, and a pharmaceutically acceptable carrier.

[0012] According to another aspect, there is provided a method for treating an NMDA receptor-related encephalopathy in a subject in need thereof, the method comprising administering to the subject a therapeutically effective amount of a recombinant AAV vector comprising: (a) a nucleic acid sequence encoding an NMDA receptor subunit; and (b) a promoter having a length ranging between 50 and 500 bp, wherein the nucleic acid sequence and the promoter are operably linked.

[0013] In some embodiments, the length of the nucleic acid sequence and of the promoter combined, ranges between 4 Kilobase pairs (Kbp) and 5 Kbp.

[0014] In some embodiments, the length of the nucleic acid sequence ranges between 4 Kbp and 5 Kbp.

[0015] In some embodiments, the nucleic acid sequence comprises a sequence encoding a wildtype (w.t.) NMDA receptor subunit.

[0016] In some embodiments, the promoter is a neuron-specific promoter.

[0017] In some embodiments, the NMDA receptor subunit is selected from the group consisting of: GluN2B, GluN1, GluN2A, GluN2C, GluN2D, GluN3A, and GluN3B.

[0018] In some embodiments, the promoter is selected from the group consisting of: mini-promoter mp-84, human synapsin (hSYN) promoter, and mini-promoter pCALM1 (mp-120).

[0019] In some embodiments, the recombinant AAV vector further comprises a post-transcriptional regulatory element.

[0020] In some embodiments, the post-transcriptional regulatory element comprises a woodchuck hepatitis virus post-transcriptional regulatory element (WPRE).

[0021] In some embodiments, the pharmaceutical composition is for use in the treatment of an NMDA receptor-related encephalopathy in a subject in need thereof.

[0022] In some embodiments, the NMDA receptor-related encephalopathy is selected from the group consisting of: GRIN1-related neurodevelopmental disorder, GRIN2A developmental disorder, GRIN2B epilepsy, GRIN2A-related epilepsy-aphasia spectrum disorders, GRIN2B-related neurodevelopmental disorder, GRIN2D-related epileptic encephalopathy, Anti-NMDA receptor encephalitis, GRIN2C-associated intellectual disability, GRIN3A-related neurodevelopmental disorder, GRIN3B-associated neurological conditions, GRIN2A-related speech disorders, GRIN1-related epileptic encephalopathy, GRIN2B-associated autism spectrum disorder, GRIN2D-related movement disorders, GRIN1-associated schizophrenia, GRIN2A-related Landau-Kleffner syndrome, and GRIN2B-related West syndrome.

[0023] In some embodiments, the administering comprises intravenously administering, intranasally administering, cerebrospinal fluid (CFS) administering, intracerebroventricularly administering, or any combination thereof.

[0024] In some embodiments, the recombinant AAV vector is in a pharmaceutical, and the pharmaceutical composition further comprises a pharmaceutically acceptable carrier, a blood-brain barrier (BBB) permeability enhancing agent, or both.

[0025] Unless otherwise defined, all technical and/or scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which the invention pertains. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of embodiments of the invention, exemplary methods and/or materials are described below. In case of conflict, the patent specification, including definitions, will control. In addition, the materials, methods, and examples are illustrative only and are not intended to be necessarily limiting.

[0026] Further embodiments and the full scope of applicability of the present invention will become apparent from the detailed description given hereinafter. However, it should be understood that the detailed description and specific examples, while indicating preferred embodiments of the invention, are given by way of illustration only, since various changes and modifications within the spirit and scope of the invention will become apparent to those skilled in the art from this detailed description.

[0027] The foregoing general description of the illustrative embodiments and the following detailed description thereof are merely exemplary aspects of the teachings of this disclosure and are not restrictive.

BRIEF DESCRIPTION OF FIGURES

[0028] Non-limiting and non-exhaustive examples are described with reference to the following figures.

[0029] **Figures 1A-1E** include graphs showing that the HA tag (a protein tag derived from the human influenza hemagglutinin- HA protein) does not affect the affinity of GluN2B-containing receptors (GluN2B receptors or NR2B receptors, in brief) towards glutamate or glycine, nor affect the receptors current amplitudes (which demonstrates no negative effect over expression levels). (**1A**) Representative glutamate-dependent currents recorded from *Xenopus* oocytes co-expressing rat GluN1a with rat GluN2B-wt (black); rat HA-GluN2B-wt (blue). Glutamate concentrations are marked next to current steps; summarized in (**1B**) and indicating no changes in glutamate affinity. (**1C**) Representative glycine-dependent currents recorded from *Xenopus* oocytes co-expressing rat GluN1a with rat GluN2B-wt (black); rat HA-GluN2B-wt (blue). Glutamate concentrations are marked next to current steps; summarized in (**1D**) and indicating no changes in glycine affinity. n.s., non-significant. (**1E**) A graph showing essentially no difference in current amplitudes of rNR2B-wt and HA-rNR2B-wt.

[0030] **Figures 2A-2H** include graphs showing that the HA-tag does not affect GluN2B sensitivity towards various allosteric modulators. (**2A**, **2C**, and **2F**) Representative

glutamate-dependent currents recorded from *Xenopus* oocytes co-expressing rat GluN1a with rat GluN2B-wt (black) or rat HA-GluN2B-wt (blue) in response to decreases in pH (**2A**) or ifenprodil (**2C**) or zinc (**2F**), respectively. pH, ifenprodil and zinc concentrations are indicated next to current steps. (**2B**, **2D-2E**, and **2G-2H**) Summary of pH sensitivity (**2B**), ifenprodil sensitivity (**2D-2E**) and zinc sensitivity (**2G-2H**), between rat GluN2B-wt and HA-GluN2B-wt, indicating no changes. n.s., non-significant.

[0031] **Figure 3** includes non-limiting schemes showing different AAV producing plasmids containing HA tagged-*GRIN2B* gene. Schematic representation of HA-tagged *GRIN2B* gene under different promotors (from top to bottom: hSYN, mp-120 and mp-84 promoters). Lengths (in base pairs, bps) of the genes, HA tag, promoters, and the chimeric intron are indicated in color coded numbers within the illustration (length of tag is noted in red within the grey bar, and length of gene in black).

[0032] **Figures 4A-4D** include fluorescent micrographs and graphs showing HA-tagged *GRIN2B* gene expression in mammalian cell line via short or mini promoters. (**4A**) Confocal image of HEK293t cells co-expressing *GRIN1a* (NR1a) and hSYN HA-r*GRIN2B-wt* (NR2B; h-human, r-rat; immuno-stained against HA (red), nuclear labeling in blue, DAPI). Surface expression of HA-rNR2B is visible (arrowhead). (**4B**) Representative currents from HEK293t cells co-expressing NR1a and NR2B under various promoters (CMV, hSYN, mp-120), summarized in (**4D**). (**4C**) Confocal image of HEK293t expressing YFP via mp-84 promoter without (– intron; ***left***) and with (+ intron; ***right***) internal chimeric intron. (***Right***) Representative currents from HEK293 cells expressing mp-84- +/-intron-HA-rNR2B-*wt* (co-expressed with *GRIN1a*), summarized in (**4D**). For brevity, lack of intron is marked as (-), while presence of intron as (+).

[0033] **Figure 5** includes fluorescent micrographs showing that DNA-transfection of Primary neurons yields expression HA-NR2B. Confocal images showing neuronal expression of NR2B on dendritic membrane and synaptic contacts (insets), following co-transfection of DNAs encoding for HA-NR2Bwt under three different promoters (hSYN,

mp-120, mp-84 with the chimeric intron; indicated by +) along soluble GFP. HA was immunolabelled (red).

[0034] **Figures 6A-6E** include fluorescent micrographs showing AAV-infection in-*vitro* of hsyn-HA-*GRIN2B* AAVs. (**6A-6C**) Representative confocal images from different experiments of primary rat hippocampal neurons infected with AAV1 expressing HA-*GRIN2B-wt* under hSYN promoter (1.3×10^{11}particles/ml); stained with an anti-HA antibody (red). (**6D**) High power image showing the expression of the protein on both sides of the dendrite (bottom inset- white arrows). (**6E**) Non-infected neurons show no immuno-labeling. All images were taken under identical settings.

[0035] **Figures 7A-7C** include photographs and a graph showing the expression of HA-tagged rNR2B under different promoters in infected hippocampal neurons. (**7A**) Presence of HA-tagged rNR2B (~175 kDa) detected by western blotting against anti-HA antibody in AAV infected hippocampal neurons. HA-rNR2B expression was driven by AAV-1 infection expressed under different promoters. Naïve (non-transfected) neurons served as a negative control. (**7B**) Corresponding blot probed with anti-β-actin antibody as a loading control. (**7C**) Quantification of HA-rNR2B expression levels normalized to β-actin (denoting amount of all cells in the well- not just neurons), showing highest expression under the hSYN promoter. Data are represented in arbitrary units (a.u.).

[0036] **Figure 8** includes photographs (of western blot analysis) and graphs showing the expression of HA-tagged rNR2B under different promoters exclusively in infected hippocampal neurons. AAV yields very high tropism towards neurons. The expression of rNR2B by the different promoters was compared, specifically in the neuronal population, by staining for beta3-tubulin (this marker labels only neurons in the well). hSYN was shown to be highly potent, followed by MP-84(+) and MP-120.

[0037] **Figure 9** includes fluorescent micrographs showing that viral delivery of HA-NR2B yields very high infection efficiency of neurons in culture. Micrographs show cultured hippocampal neurons infected with AAV1-HArNR2B. These demonstrate very high neuronal infection efficiency, e.g., # of neurons expressing HA-rNR2B (red; ***Left***)

from the total amount of neurons in the dish (labeled by anti-MAP2, green; ***Middle***). Note that almost all neurons (green) are expressing HA-rNR2B (red). There is no expression outside neurons. ***Right*** image represents the merge of ***Left*** and ***Middle*** images.

[0038] **Figures 10A-10C** include an illustration and fluorescent micrographs showing *in-vivo* expression of hsyn-HA-*GRIN2B*. (**10A**) A cartoon representation of tail vein-injection of AAV9 CAP-B10-hSYN–HA-rat-*Grin2Bwt* in mice. (**10B**) A confocal image of a 50 mm brain slice (showing a large portion of the right cortex). (**10C**) High power image showing individual neurons and processes. The inventors injected a third of the standard volume used by others. Rhodamine immunostaining against HA (red).

[0039] **Figure 11** includes fluorescent micrographs and a graph showing that the overexpression of HA-rNR2B in cultured neurons does not induce excitotoxicity. Shown are confocal micrographs of cultured neurons infected AAV1 to express HA-tagged rNR2B under various promoters (red), followed by TUNEL labeling (green) to determine levels of excitotoxicity. Two positive controls for toxicity were used- DNAse-treatment and exposure of neurons to the ligand NMDA (100 μM). The positive controls caused massive neuronal death (seen as bright green nuclei in micrographs). Expression of the HA-rNR2A under various promoters does not cause cell death, compared to negative control- mock infection (AAV1 expressing a red fluorescent protein; tdtomato). Summary at bottom right.

[0040] **Figure 12A-12E** include graphs and a table showing that heterozygous *Grin2B* knock-out (KO) rats do not display altered behavior in select behavioral tests. (**12A**) Social cooperation test: The average rewards obtained by *wt* (blue) and heterozygous (red) rats over 18 days. (**12B**) The startle reaction (force in Newtons, N) of *wt* (blue) and heterozygous (red) rats across increasing sound intensities (dB). Both groups exhibited similar response patterns. (**12C**) Rotarod test: Latency to fall (in seconds) over three days of testing. (**12D**) Open field test: Total distance moved (centimeters) in an open field arena. *wt* (blue) and heterozygous (red) rats showed comparable motor learning and coordination. The results further show differences in animals' weight (**12E**).

[0041] **Figure 13A-13K** includes graphs showing the characterization of cultured neurons from *Grin2B+/G689C mice.* (**13A**) Representative traces of action potentials recorded from Grin2B+/+ (black) and Grin2B+/G689C (purple). (**13B-13G**) Quantification of intrinsic neuronal properties. All results were non-significant expect for input resistance, showing a significant increase ($p < 0.01$) in Grin2B+/G689C neurons. h. Number of action potentials (AP) in response to increasing current injections, demonstrating no significant differences between genotypes. i. Representative traces of voltage-gated sodium currents recorded from Grin2B+/+ (black) and Grin2B+/G689C (purple). (**13J-13K**). I-V curves of fast sodium (**13J**) and fast potassium currents (**13K**) Shows no differences between genotypes. Error bars represent standard error of the mean (SEM). n.s. = not significant, ** $p < 0.01$. N=2, n=21-23 neurons.

[0042] **Figure 14A-14D** includes graphs showing that neurons from *Grin2B*$^{+/G689C}$- mice display reduced NMDAR sEPSCs amplitude. **(14A-14B)** Electrophysiological recordings from neurons produced from Grin2B$^{+/+}$ (**14A**) and Grin2B$^{+/G689C}$ (**14B**) sibling mice. Representative recordings show marked differences between the neurons in NMDAR-EPSC. AMPAR and NMDAR sEPSCs amplitude summarized in (**14C**) and (**14D**) respectively showing significant reduction in NMDAR sEPSCs amplitude.

[0043] **Figures 15A-15F** include an image and graphs showing of Grin2B knock-in (KI) female mice showing reduced Long-Term Potentiation (LTP in slice). (**15A**) Left – Micrograph of mouse brain slice during recording of fEPSPs. Dashed black lines indicate placement of the recording electrode in the Stratum Radiatum of CA1. Right – Averaged traces of fEPSP recordings either *Grin2B*$^{+/+}$ (black) or *Grin2B*$^{+/G689C}$ (purple) mice. Grey traces show fEPSPs after theta burst induction. (**15B-15D**) Summary of the change over time in fEPSP slope before and after theta burst induction in both sexes (**15B**), females alone (**15C**) and male alone (**15D**). (**15E**) Summary of the average change in fEPSP slope following theta burst induction of both sexes combined. (**15F**) Summary of the average change in fEPSP slope following theta burst induction divided into different sexes. Error bars represent standard error of the mean (SEM). n.s. = not significant, * $p < 0.05$.

[0044] **Figure 16A-16D** include graphs showing that heterozygous *Grin2B* knock-in (KI) mice do not display altered behavior. (**16A**) Activity cage test: Nighttime locomotor activity of *wt* and Het mice over 7 days, measured as activity counts. (**16B**) Latency to fall (seconds) in *wt* and Het mice, assessing motor coordination and balance. (**16C**) Open field test: Total distance moved (meters) in an open field arena. (**16D**) Morris water maze test: Latency (seconds) to find the hidden platform, evaluating spatial learning and memory. In all panels *wt* (black) and heterozygous (purple). In all comparative tests, there was not statistical significance; N=1-2, n=3-6.

[0045] **Figure 17A-17B** include fluorescent micrographs showing AAV-infection in-*vitro* of HA-*Grin1a or* HA-*Grin2D* AAVs. (**17A-17B**) Representative confocal images of permeabilized primary rat hippocampal neurons infected with AAV1 expressing HA-*Grin1a-wt* (**17A**) or HA-*Grin2D-wt* (**17B**); stained with an anti-HA antibody (orange). These demonstrate the feasibility of successfully packaging other NMDAR-subunits and producing functional and infectious AAV particles (thus paving the way towards packaging other large, e.g., receptor, subunits within AAVs).

DETAILED DESCRIPTION

[0046] The following description sets forth exemplary aspects of the present disclosure. It should be recognized, however, that such description is not intended as a limitation on the scope of the present disclosure. Rather, the description also encompasses combinations and modifications to those exemplary aspects described herein.

Recombinant AAV

[0047] According to the first aspect, there is provided a recombinant adeno-associated virus (AAV) vector comprising: (a) a nucleic acid sequence encoding an N-methyl-D-aspartate (NMDA) receptor subunit; and (b) a promoter having a length ranging between 50 and 500 bp.

[0048] In some embodiments, the nucleic acid sequence and the neuron specific promoter are operably linked.

[0049] In some embodiments, the promoter is a neuro-specific promoter.

[0050] As used herein, the term "neuron-specific promoter" may refer to a regulatory DNA sequence that directs gene expression predominantly or exclusively in neurons. These promoters may initiate transcription of downstream genes in neuronal cells while remaining largely inactive in non-neuronal cell types. Neuron-specific promoters may be derived from genes that are naturally expressed primarily in neurons, or they may be engineered or modified versions of such promoters. These promoters may vary in their specificity, with some being active in broad neuronal populations and others restricted to particular neuronal subtypes. The activity of neuron-specific promoters may be influenced by developmental stage, brain region, or specific neuronal activation states. In the context of gene therapy or transgenic approaches, neuron-specific promoters may be used to target gene expression to neurons while minimizing expression in other cell types.

[0051] In some embodiments, the promoter further comprises a non-coding nucleic acid sequence. In some embodiments, the promoter is ligated or conjugated to a non-coding nucleic acid sequence. In some embodiments, the promoter is ligated or conjugated to an intron or a fragment thereof. In some embodiments, the length of the intron or a fragment thereof is 10-200 bp, 20-200 bp, 50-200 bp, 100-200 bp, or 50-250 bp. Each possibility represents a separate embodiment of the invention. In some embodiments, the intron comprises the nucleic acid sequence set forth in SEQ I NO: 5, or a functional analog thereof having at least 80%, 90%, 95%, or 99% homology/identity thereto, or any value and range therebetween. Each possibility represents a separate embodiment of the invention. In some embodiments, the intron comprises the nucleic acid sequence set forth in SEQ ID NO: 5.

[0052] In some embodiments, the promoter and the non-coding nucleic acid sequence are derived from the same gene. In some embodiments, the promoter and the non-coding nucleic acid sequence are derived from distinct genes.

[0053] As used herein, the term "operably linked" may refer to a functional relationship between two or more nucleic acid sequences in which the components are arranged in a manner that allows them to function together for their intended purpose. In the context of gene expression, operably linked sequences may typically include a promoter and a coding

sequence, where the promoter is positioned to direct the transcription of the coding sequence. This arrangement may allow for the proper expression of the gene product encoded by the coding sequence. Operably linked elements may be contiguous or non-contiguous on a nucleic acid molecule, and may include additional regulatory elements such as enhancers, silencers, or terminators. The functional relationship between operably linked sequences may be maintained when they are incorporated into a vector or other genetic construct.

[0054] In some embodiments, the length of a nucleic acid sequence and of a promoter combined, ranges between 4 Kilobase pairs (Kbp) and 5 Kbp, 3.9 Kbp and 5.2 Kbp, 3.7 Kbp and 5.5 Kbp, 4.1 Kbp and 5.2 Kbp, 4.2 Kbp and 5.0 Kbp, 4.3 Kbp and 5.0 Kbp, 4.5 Kbp and 5.5 Kbp, 4.4 Kbp and 5.1 Kbp, 4.5 Kbp and 5.3 Kbp, 4.5 Kbp and 5.1 Kbp. Each possibility represents a separate embodiment of the invention.

[0055] In some embodiments, the nucleic acid sequence comprises a sequence of a wildtype (w.t.) NMDA receptor subunit. In some embodiments, the nucleic acid sequence consists of a sequence of a wildtype (w.t.) NMDA receptor subunit. In some embodiments, w.t. is human w.t. In some embodiments, the nucleic acid sequence is codon optimized for expression in a human or a cell thereof. In some embodiments, a cell is a neuron cell. In some embodiments, the nucleic acid sequence comprises a sequence encoding a wildtype (w.t.) NMDA receptor subunit. In some embodiments, the nucleic acid sequence encoding a wildtype (w.t.) NMDA receptor subunit comprises at least one silent or a synonymous mutation.

[0056] As used herein, the term "codon optimized" may refer to a process of modifying the nucleotide sequence of a gene to enhance its expression in a particular host organism or cell type, without altering the amino acid sequence of the encoded protein. This optimization may involve replacing rare codons with more frequently used synonymous codons preferred by the host's translational machinery. Codon optimization may take into account factors such as codon usage bias, GC content, mRNA secondary structure, and regulatory sequences. The goal of codon optimization may be to improve translational

efficiency, increase protein yield, or enhance mRNA stability. In some cases, codon optimization may also involve adjusting other parameters that can affect gene expression, such as avoiding repetitive sequences or potential splice sites.

[0057] As used herein, the term "NMDA receptor subunit" may refer to a protein component that forms part of the N-methyl-D-aspartate (NMDA) receptor complex. NMDA receptors are ionotropic glutamate receptors found in neural cells, composed of multiple subunits that assemble to form functional ion channels. These subunits may include, but are not limited to, GluN1, GluN2A, GluN2B, GluN2C, GluN2D, GluN3A, and GluN3B. Each subunit may contribute specific properties to the receptor complex, influencing its pharmacological and electrophysiological characteristics. NMDA receptor subunits may be encoded by distinct genes and can undergo alternative splicing, potentially resulting in multiple isoforms. The composition and expression of these subunits may vary across different brain regions, developmental stages, and in response to various physiological or pathological conditions.

[0058] In some embodiments, the length of a promoter ranges between 50 bp and 500 bp, 70 bp and 500 bp, 100 bp and 500 bp, 150 bp and 500 bp, 200 bp and 500 bp, 250 bp and 500 bp, 300 bp and 500 bp, 350 bp and 500 bp, 400 bp and 500 bp, 450 bp and 500 bp, 50 bp and 400 bp, 60 bp and 450 bp, 70 bp and 350 bp, 50 bp and 350 bp, 70 bp and 320 bp, or 70 bp and 200 bp. Each possibility represents a separate embodiment of the invention.

[0059] In some embodiments, the NMDA receptor subunit is selected from: GluN2B, GluN1, GluN2A, GluN2C, GluN2D, GluN3A, or GluN3B. In some embodiments, the NMDA receptor subunit is GluN2B. In some embodiments, the NMDA receptor subunit is GluN1. In some embodiments, the NMDA receptor subunit is GluN2A. In some embodiments, the NMDA receptor subunit is GluN2C. In some embodiments, the NMDA receptor subunit is GluN2D. In some embodiments, the NMDA receptor subunit is GluN3A. In some embodiments, the NMDA receptor subunit comprises GluN2B. In some embodiments, the NMDA receptor subunit comprises GluN1. In some embodiments, the NMDA receptor subunit comprises GluN2A. In some embodiments, the NMDA receptor

subunit comprises GluN2C. In some embodiments, the NMDA receptor subunit comprises GluN2D. In some embodiments, the NMDA receptor subunit comprises GluN3A.

[0060] As used herein, the term "GluN2B" may refer to a specific subunit of the N-methyl-D-aspartate (NMDA) receptor, also known as the NR2B subunit or GRIN2B. GluN2B is a protein encoded by the GRIN2B gene in humans. This subunit may play a crucial role in the formation and function of NMDA receptors, which are involved in synaptic plasticity, learning, and memory processes in the central nervous system. GluN2B may contribute to the receptor's unique properties, including its voltage-dependent magnesium block, high calcium permeability, and slow deactivation kinetics. The expression and distribution of GluN2B may vary during development and across different brain regions. Alterations in GluN2B function or expression have been associated with various neurological and psychiatric disorders. The accession number for the human GluN2B (GRIN2B) gene is NM_000834.4. This accession number corresponds to the NCBI Reference Sequence for the mRNA transcript of the GRIN2B gene. The protein encoded by this gene has the accession number NP_000825.2.

[0061] In some embodiments, the nucleic acid sequence encoding GluN2B comprises the nucleic acid sequence set forth in SEQ ID NO: 1, or a functional analog thereof having at least 80%, 90%, 95%, or 99% homology/identity thereto, or any value and range therebetween. Each possibility represents a separate embodiment of the invention. In some embodiments, the nucleic acid sequence encoding GluN2B consists of the nucleic acid sequence set forth in SEQ ID NO: 1.

[0062] As used herein, the term "GluN1" may refer to a specific subunit of the N-methyl-D-aspartate (NMDA) receptor, also known as the NR1 subunit or GRIN1. GluN1 is a protein encoded by the GRIN1 gene in humans. This subunit is considered an obligatory component of functional NMDA receptors, as it is required for the formation of the receptor complex. GluN1 may be present in all NMDA receptor subtypes and can combine with various GluN2 or GluN3 subunits to form heteromeric receptors. The GluN1 subunit may contain the binding site for glycine, a co-agonist necessary for NMDA receptor activation.

Multiple splice variants of GluN1 may exist, potentially contributing to the functional diversity of NMDA receptors. The expression and regulation of GluN1 may be critical for normal neuronal function and synaptic plasticity. The accession number for the human GluN1 (GRIN1) gene is NM_007327.4. This accession number corresponds to the NCBI Reference Sequence for the mRNA transcript of the GRIN1 gene. The protein encoded by this gene has the accession number NP_015566.1.

[0063] As used herein, the term "GluN2A" may refer to a specific subunit of the N-methyl-D-aspartate (NMDA) receptor, also known as the NR2A subunit or GRIN2A. GluN2A is a protein encoded by the GRIN2A gene in humans. This subunit may contribute to the formation of functional NMDA receptors by assembling with GluN1 subunits. GluN2A may play a role in determining the biophysical and pharmacological properties of the NMDA receptor complex, including its kinetics, conductance, and sensitivity to modulators. The expression of GluN2A may vary during development and across different brain regions, with its levels generally increasing postnatally. GluN2A-containing NMDA receptors may be involved in various aspects of synaptic plasticity, learning, and memory. Mutations in the GRIN2A gene have been associated with certain neurodevelopmental disorders and epilepsy syndromes. The accession number for the human GluN2A (GRIN2A) gene is NM_000833.5. This accession number corresponds to the NCBI Reference Sequence for the mRNA transcript of the GRIN2A gene. The protein encoded by this gene has the accession number NP_000824.1.

[0064] As used herein, the term "GluN2C" may refer to a specific subunit of the N-methyl-D-aspartate (NMDA) receptor, also known as the NR2C subunit or GRIN2C. GluN2C is a protein encoded by the GRIN2C gene in humans. This subunit may combine with GluN1 subunits to form functional NMDA receptors with distinct properties. GluN2C-containing NMDA receptors may exhibit lower conductance, reduced magnesium sensitivity, and slower deactivation kinetics compared to receptors containing other GluN2 subunits. The expression of GluN2C may be more restricted compared to other NMDA receptor subunits, with higher levels typically found in the cerebellum, thalamus, and olfactory bulb. GluN2C

may play roles in cerebellar function, motor coordination, and certain aspects of sensory processing. The unique properties of GluN2C-containing receptors may contribute to specialized neuronal signaling in the regions where it is expressed. The accession number for the human GluN2C (GRIN2C) gene is NM_000835.5. This accession number corresponds to the NCBI Reference Sequence for the mRNA transcript of the GRIN2C gene. The protein encoded by this gene has the accession number NP_000826.2.

[0065] As used herein, the term "GluN2D" may refer to a specific subunit of the N-methyl-D-aspartate (NMDA) receptor, also known as the NR2D subunit or GRIN2D. GluN2D is a protein encoded by the GRIN2D gene in humans. This subunit may assemble with GluN1 subunits to form functional NMDA receptors with distinct physiological and pharmacological properties. GluN2D-containing NMDA receptors may be characterized by prolonged channel opening times, low conductance, and reduced sensitivity to magnesium block compared to receptors containing other GluN2 subunits. The expression of GluN2D may be highest during early development and in specific brain regions such as the diencephalon, brainstem, and spinal cord. GluN2D may play roles in neonatal brain development, pain processing, and certain aspects of motor function. The unique properties of GluN2D-containing receptors may contribute to specialized neuronal signaling in the regions and developmental stages where it is predominantly expressed. The accession number for the human GluN2D (GRIN2D) gene is NM_000836.3. This accession number corresponds to the NCBI Reference Sequence for the mRNA transcript of the GRIN2D gene. The protein encoded by this gene has the accession number NP_000827.2.

[0066] As used herein, the term "GluN3A" may refer to a specific subunit of the N-methyl-D-aspartate (NMDA) receptor, also known as the NR3A subunit or GRIN3A. GluN3A is a protein encoded by the GRIN3A gene in humans. This subunit may assemble with GluN1 and GluN2 subunits to form functional NMDA receptors with unique properties. GluN3A-containing NMDA receptors may exhibit reduced calcium permeability, decreased magnesium sensitivity, and altered channel kinetics compared to conventional NMDA receptors. The expression of GluN3A may be highest during early postnatal development

and may decrease in adulthood, with some regional variations. GluN3A may play roles in synaptic plasticity, dendritic spine development, and neuroprotection. The incorporation of GluN3A into NMDA receptors may modulate their function, potentially influencing neuronal excitability and synaptic transmission during critical periods of brain development. The accession number for the human GluN3A (GRIN3A) gene is NM_133445.4. This accession number corresponds to the NCBI Reference Sequence for the mRNA transcript of the GRIN3A gene. The protein encoded by this gene has the accession number NP_597702.2.

[0067] As used herein, the term "GluN3B" may refer to a specific subunit of the N-methyl-D-aspartate (NMDA) receptor, also known as the NR3B subunit or GRIN3B. GluN3B is a protein encoded by the GRIN3B gene in humans. This subunit may combine with GluN1 and GluN2 subunits to form functional NMDA receptors with distinct properties. GluN3B-containing NMDA receptors may exhibit reduced calcium permeability and altered channel kinetics compared to conventional NMDA receptors. The expression of GluN3B may be more restricted compared to other NMDA receptor subunits, with higher levels typically found in motor neurons of the spinal cord and brainstem. GluN3B may play roles in motor neuron function, neuroprotection, and modulation of excitatory neurotransmission. The incorporation of GluN3B into NMDA receptors may contribute to specialized signaling properties in the regions where it is expressed. The accession number for the human GluN3B (GRIN3B) gene is NM_138690.4. This accession number corresponds to the NCBI Reference Sequence for the mRNA transcript of the GRIN3B gene. The protein encoded by this gene has the accession number NP_619635.1.

[0068] In some embodiments, the promoter is selected from: mini-promoter mp-84, human synapsin (hSYN) promoter, and mini-promoter pCALM1 (mp-120).

[0069] In some embodiments, the promoter comprises hSYN. In some embodiments, the promoter consists of hSYN. In some embodiments, the promoter being hSYN comprises the nucleic acid sequence set forth in SEQ ID NO: 2. In some embodiments, the promoter being hSYN consists of the nucleic acid sequence set forth in SEQ ID NO: 2.

[0070] In some embodiments, the promoter comprises mini-promoter mp-84. In some embodiments, the promoter consists of mini-promoter mp-84. In some embodiments, the promoter being mini-promoter mp-84 comprises the nucleic acid sequence set forth in SEQ ID NO: 3. In some embodiments, the promoter being mini-promoter mp-84 consists of the nucleic acid sequence set forth in SEQ ID NO: 3.

[0071] In some embodiments, the promoter comprises mp-120. In some embodiments, the promoter consists of mp120. In some embodiments, the promoter comprises mp120. In some embodiments, the promoter consists of mp120. In some embodiments, the promoter being mp120comprises the nucleic acid sequence set forth in SEQ ID NO: 4. In some embodiments, the promoter being mp120 consists of the nucleic acid sequence set forth in SEQ ID NO: 4.

[0072] In some embodiments, the recombinant AAV vector of the invention further comprises a post-transcriptional regulatory element.

[0073] As used herein, the term "post-transcriptional regulatory element" may refer to a nucleic acid sequence that influences gene expression after transcription has occurred. This element may be located in the untranslated regions (UTRs) of mRNA or within the coding sequence itself. Post-transcriptional regulatory elements may affect various aspects of mRNA processing, stability, localization, or translation efficiency. These elements may interact with specific RNA-binding proteins or other regulatory factors to modulate gene expression. Types of AAV post-transcriptional regulatory elements are common and would be apparent to one of ordinary skill in the art. Non-limiting examples of such post-transcriptional regulatory elements include, but are not limited to, internal ribosome entry sites (IRES), AU-rich elements (AREs), and elements that affect splicing, polyadenylation, or mRNA decay. In some cases, post-transcriptional regulatory elements may be derived from viral genomes, such as the Woodchuck Hepatitis Virus Post-transcriptional Regulatory Element (WPRE), which may enhance gene expression by increasing mRNA stability and nuclear export.

[0074] In some embodiments, the post-transcriptional regulatory element comprises a woodchuck hepatitis virus post-transcriptional regulatory element (WPRE). In some embodiments, the WPRE comprises the nucleic acid sequence set forth in SEQ ID NO: 6. In some embodiments, the WPRE consists of the nucleic acid sequence set forth in SEQ ID NO: 6.

[0075] In some embodiments, the recombinant AAV vector of the invention comprises a pair of inverted terminal repeats (ITRs). In some embodiments, the pair of ITRs flank the: (a) nucleic acid sequence encoding an N-methyl-D-aspartate (NMDA) receptor subunit; and (b) neuron-specific promoter nucleic acid sequence. In some embodiments, the pair of IRTs comprises a first ITR and a second ITR.

[0076] In some embodiments, the organization of nucleic acid sequence of the recombinant AAV vector of the invention is (from 5' to 3'): first ITR, neuron-specific promoter nucleic acid sequence, nucleic acid sequence encoding an N-methyl-D-aspartate (NMDA) receptor subunit, and a second ITR.

[0077] In some embodiments, the first ITR comprises the nucleic acid sequence set forth in SEQ ID NO: 7. In some embodiments, the first ITR consists of the nucleic acid sequence set forth in SEQ ID NO: 7.

[0078] In some embodiments, the second ITR comprises the nucleic acid sequence set forth in SEQ ID NO: 8. In some embodiments, the second ITR consists of the nucleic acid sequence set forth in SEQ ID NO: 8.

[0079] As used herein, the term "inverted terminal repeat (ITR)" may refer to a specific DNA sequence found at both ends of a viral genome or vector. ITRs are characterized by their palindromic nature, meaning the sequence reads the same in both directions. In the context of adeno-associated virus (AAV) vectors, ITRs may be crucial structural elements that flank the transgene cassette. These sequences may play important roles in viral replication, packaging of the viral genome into capsids, and integration into the host genome. ITRs may also serve as primers for DNA synthesis during viral replication. In some cases, ITRs may enhance gene expression by promoting nuclear transport of the

vector genome. The presence of ITRs may be essential for the functionality of AAV-based gene therapy vectors, as they may be required for efficient transgene expression and vector production. In some embodiments, the length of a nucleic acid sequence and of a promoter combined, ranging between 4 Kilobase pairs (Kbp) and 5 Kbp is determined between the ITRs. In some embodiments, the distance between the ITRs of the AAV is ranging between 4 Kbp and 5.2 Kbp. In some embodiments, the distance between the ITRs of the AAV ranging between 4 Kbp and 5.2 Kbp is occupied by the nucleic acid sequence and the promoter, of the invention.

Pharmaceutical composition

[0080] According to another aspect, there is provided a composition comprising the recombinant AAV vector of the invention. In some embodiments, the composition comprises a therapeutically effective amount of the recombinant AAV vector of the invention. In some embodiments, the composition further comprises a pharmaceutically acceptable carrier. In some embodiments, the composition is a pharmaceutical composition.

[0081] As used herein, the term "carrier" refers to a diluent, adjuvant, excipient, or vehicle with which the therapeutic compound is administered. Such pharmaceutical carriers can be sterile liquids, such as water and oils, including those of petroleum, animal, vegetable or synthetic origin, such as peanut oil, soybean oil, mineral oil, sesame oil and the like, polyethylene glycols, glycerin, propylene glycol or other synthetic solvents. Water is a preferred carrier when the pharmaceutical composition is administered intravenously. Saline solutions and aqueous dextrose and glycerol solutions can also be employed as liquid carriers, particularly for injectable solutions. Suitable pharmaceutical excipients include starch, glucose, lactose, sucrose, gelatin, malt, rice, flour, chalk, silica gel, sodium stearate, glycerol monostearate, talc, sodium chloride, dried skim milk, glycerol, propylene glycol, water, ethanol and the like. The composition, if desired, can also contain minor amounts of wetting or emulsifying agents, or pH buffering agents such as acetates, citrates or phosphates. Antibacterial agents such as benzyl alcohol or methyl parabens; antioxidants

such as ascorbic acid or sodium bisulfite; and agents for the adjustment of tonicity such as sodium chloride or dextrose are also envisioned. The carrier may comprise, in total, from about 0.1% to about 99.99999% by weight of the pharmaceutical compositions presented herein.

[0082] As used herein, the term "pharmaceutically acceptable" means suitable for administration to a subject, e.g., a human. For example, the term "pharmaceutically acceptable" can mean approved by a regulatory agency of the Federal or a state government or listed in the U. S. Pharmacopeia or other generally recognized pharmacopeia for use in animals, and more particularly in humans.

[0083] In another embodiment, the compositions of the invention take the form of solutions, suspensions, emulsions, sustained-release formulations and the like. Examples of suitable pharmaceutical carriers are described in: Remington's Pharmaceutical Sciences" by E.W. Martin, the contents of which are hereby incorporated by reference herein. Such compositions will contain a therapeutically effective amount of the recombinant AAV vector of the invention, preferably in a substantially purified form, together with a suitable amount of carrier so as to provide the form for proper administration to the subject.

[0084] According to an embodiment of the invention, pharmaceutical compositions contain 0.1-95% of the recombinant AAV vector of the invention. According to another embodiment of the invention, pharmaceutical compositions contain 1-70% of the recombinant AAV. According to another embodiment of the invention, the composition or formulation to be administered may contain a quantity of recombinant AAV, according to embodiments of the invention in an amount effective to treat the condition or disease of the subject being treated.

[0085] An embodiment of the invention relates to a recombinant AAV vector of the invention, presented in unit dosage form and prepared by any one of the methods well known in the art of pharmacy. In addition, in vitro assays may optionally be employed to help identify optimal dosage ranges. The precise dose to be employed in the formulation will also depend on the route of administration, and the nature of the disease or disorder,

and should be decided according to the judgment of the practitioner and each patient's circumstances. Effective doses can be extrapolated from dose-response curves derived from in-vitro or in-vivo animal model test bioassays or systems.

[0086] According to one embodiment, the compositions of the invention are administered in the form of a pharmaceutical composition comprising an active component/ingredient of this invention (the recombinant AAV) together with a pharmaceutically acceptable carrier or diluent. In another embodiment, the compositions of this invention can be administered either individually or together in any conventional intravenous, intranasal, or intracerebroventricular (ICV) dosage form. In some embodiments, the pharmaceutical composition further comprises at least one blood-brain barrier (BBB) permeability enhancing agent. In some embodiments, the pharmaceutical composition is adopted for combined administration with a blood-brain barrier (BBB) permeability enhancing agent.

[0087] As used herein, the term "blood-brain barrier (BBB) permeability enhancing agent" may refer to a substance or compound that increases the ability of molecules, such as therapeutic agents or viral vectors, to cross the blood-brain barrier. The blood-brain barrier is a highly selective semipermeable border of endothelial cells that prevents solutes in the circulating blood from non-selectively crossing into the extracellular fluid of the central nervous system. BBB permeability enhancing agents may work through various mechanisms, such as temporarily disrupting tight junctions between endothelial cells, modulating transport proteins, or altering the physicochemical properties of the BBB. These agents may include, but are not limited to, hyperosmolar solutions, vasoactive agents, nanoparticles, peptides, or small molecules designed to interact with specific receptors or transporters at the BBB. The use of BBB permeability enhancing agents may improve the delivery of therapeutic compounds or gene therapy vectors to the brain, potentially enhancing the efficacy of treatments for neurological disorders.

[0088] Various types of agents that may enhance BBB permeability through different mechanisms, including osmotic disruption, receptor-mediated transport, and temporary

opening of tight junctions, are common and would be apparent to one of ordinary skill in the art.

[0089] Non-limiting examples of BBB permeability enhancing agents include, but are not limited to, Mannitol, Focused ultrasound, Borneol, Short-chain alkylglycerols, Bradykinin, RMP-7 (lobradimil), Adenosine, Histamine, Nitric oxide donors, Matrix metalloproteinase-9 (MMP-9), Hypertonic solutions, Sodium caprate, Claudin-5 siRNA, Regadenoson, Lexiscan (regadenoson), Cereport (RMP-7), Hyperosmolar arabinose, Microbubble-enhanced focused ultrasound, Angiopep-2 peptide, Transferrin receptor-targeting antibodies, Low-intensity pulsed ultrasound (LIPUS), Glutathione PEGylated liposomes, Borneol-modified nanoparticles, TAT peptide, and Poloxamer 188, to name a few.

[0090] In some embodiments, the composition of the invention is used in the treatment of an NMDA receptor-related encephalopathy in a subject in need thereof.

[0091] In some embodiments, an NMDA receptor-related encephalopathy is selected from: GRIN1-related neurodevelopmental disorder, GRIN2A developmental disorder, GRIN2B epilepsy, GRIN2A-related epilepsy-aphasia spectrum disorders, GRIN2B-related neurodevelopmental disorder, GRIN2D-related epileptic encephalopathy, Anti-NMDA receptor encephalitis, GRIN2C-associated intellectual disability, GRIN3A-related neurodevelopmental disorder, GRIN3B-associated neurological conditions, GRIN2A-related speech disorders, GRIN1-related epileptic encephalopathy, GRIN2B-associated autism spectrum disorder, GRIN2D-related movement disorders, GRIN1-associated schizophrenia, GRIN2A-related Landau-Kleffner syndrome, GRIN2B-related West syndrome, or any combination thereof.

[0092] As used herein, the term "NMDA receptor-related encephalopathy" may refer to a group of neurological disorders characterized by dysfunction or dysregulation of N-methyl-D-aspartate (NMDA) receptors in the brain. These conditions may result from genetic mutations affecting NMDA receptor subunits, autoimmune responses targeting NMDA receptors, or other factors that disrupt normal NMDA receptor function. NMDA

receptor-related encephalopathies may manifest with a range of symptoms, which may include seizures, cognitive impairment, developmental delays, movement disorders, behavioral changes, or altered consciousness. The severity and specific clinical presentation may vary depending on the underlying cause and the extent of NMDA receptor dysfunction. This term may encompass various disorders associated with different NMDA receptor subunit genes, such as GRIN1, GRIN2A, GRIN2B, GRIN2C, GRIN2D, GRIN3A, and GRIN3B, as well as conditions like anti-NMDA receptor encephalitis.

[0093] In some embodiments, there is provided a combination therapy for use in the treatment of NMDA receptor-related encephalopathy, the combination comprise a therapeutically effective amount of a first pharmaceutical composition comprising the recombinant AAV vector of the invention, and second pharmaceutical composition comprising a BBB permeability enhancing agent.

[0094] In some embodiments, the first and second pharmaceutical compositions are administered sequentially or concomitantly (e.g., together).

Method of treatment

[0095] According to another aspect, there is provided a method for treating an NMDA receptor-related encephalopathy in a subject in need thereof.

[0096] In some embodiments, the method comprises administering to the subject a therapeutically effective amount of a recombinant AAV vector comprising: (a) a nucleic acid sequence encoding an NMDA receptor subunit; and (b) a promoter having a length ranging between 50 and 500 bp. In some embodiments, the nucleic acid sequence and the neuron specific promoter are operably linked.

[0097] In some embodiments, administering comprises intravenously administering, intranasally administering, cerebrospinal fluid (CFS) administering, intracerebroventricularly administering, or any combination thereof. In some embodiments, administering comprises intranasally administering. In some embodiments, administering comprises intravenously administering and intranasally administering. In

some embodiments, administering comprises cerebrospinal fluid (CFS) administering. In some embodiments, administering comprises intracerebroventricularly administering.

[0098] In some embodiments, the recombinant AAV is formulated for intranasal administration. In some embodiments, the recombinant AAV is formulated for intravenous administration.

[0099] In some embodiments, the recombinant AAV vector is in a pharmaceutical, and wherein the pharmaceutical composition further comprises a pharmaceutically acceptable carrier, a blood-brain barrier (BBB) permeability enhancing agent, or both.

[0100] In some embodiments, the treating comprises administering to the subject a therapeutically effective amount of a first pharmaceutical composition comprising the recombinant AAV vector of the invention, and second pharmaceutical composition comprising a BBB permeability enhancing agent.

[0101] The term "subject" as used herein refers to an animal, more particularly to non-human mammals and human organism. Non-human animal subjects may also include prenatal forms of animals, such as, e.g., embryos or fetuses. Non–limiting examples of non-human animals include: horse, cow, camel, goat, sheep, dog, cat, non-human primate, mouse, rat, rabbit, hamster, guinea pig, pig. In one embodiment, the subject is a human subject. Human subjects may also include fetuses. In one embodiment, a subject in need thereof is a subject afflicted with and/or at risk of being afflicted with an NMDA receptor-related encephalopathy.

[0102] As used herein, the terms “treatment” or “treating” of a disease, disorder, or condition encompasses alleviation of at least one symptom thereof, a reduction in the severity thereof, or inhibition of the progression thereof. Treatment need not mean that the disease, disorder, or condition is totally cured. To be an effective treatment, a useful composition herein needs only to reduce the severity of a disease, disorder, or condition, reduce the severity of symptoms associated therewith, or provide improvement to a patient or subject’s quality of life.

[0103] As used herein, the term “prevention” of a disease, disorder, or condition encompasses the delay, prevention, suppression, or inhibition of the onset of a disease, disorder, or condition. As used in accordance with the presently described subject matter, the term "prevention" relates to a process of prophylaxis in which a subject is exposed to the presently described peptides prior to the induction or onset of the disease/disorder process. This could be done where an individual has a genetic pedigree indicating a predisposition toward occurrence of the disease/disorder to be prevented. For example, this might be true of an individual whose ancestors show a predisposition toward certain types of, for example, inflammatory disorders. The term "suppression" is used to describe a condition wherein the disease/disorder process has already begun but obvious symptoms of the condition have yet to be realized. Thus, the cells of an individual may have the disease/disorder, but no outside signs of the disease/disorder have yet been clinically recognized. In either case, the term prophylaxis can be applied to encompass both prevention and suppression. Conversely, the term "treatment" refers to the clinical application of active agents to combat an already existing condition whose clinical presentation has already been realized in a patient.

[0104] In some embodiments, treating comprises preventing and treating. In some embodiments, treating comprises ameliorating one or more symptoms associated with an NMDA receptor-related encephalopathy. In some embodiments, treating comprises ameliorating at least one symptom associated with an NMDA receptor-related encephalopathy.

[0105] As used herein, “one or more” encompasses a plurality. In some embodiments, a plurality constitutes any integer being equal to or greater than 2.

[0106] As used herein, the term "condition" includes anatomic and physiological deviations from the normal that constitute an impairment of the normal state of the living animal or one of its parts, that interrupts or modifies the performance of the bodily functions.

[0107] Any concentration ranges, percentage range, or ratio range recited herein are to be understood to include concentrations, percentages or ratios of any integer within that range and fractions thereof, such as one tenth and one hundredth of an integer, unless otherwise indicated.

[0108] Any number range recited herein relating to any physical feature, such as polymer subunits, size or thickness, are to be understood to include any integer within the recited range, unless otherwise indicated.

[0109] As used herein, the terms “subject” or “individual” or “animal” or “patient” or “mammal,” refers to any subject, particularly a mammalian subject, for whom therapy is desired, for example, a human.

[0110] In the discussion unless otherwise stated, adjectives such as “substantially” and “about” modifying a condition or relationship characteristic of a feature or features of an embodiment of the invention, are understood to mean that the condition or characteristic is defined to within tolerances that are acceptable for operation of the embodiment for an application for which it is intended. Unless otherwise indicated, the word “or” in the specification and claims is considered to be the inclusive “or” rather than the exclusive or, and indicates at least one of, or any combination of items it conjoins.

[0111] It should be understood that the terms “a” and “an” as used above and elsewhere herein refer to “one or more” of the enumerated components. It will be clear to one of ordinary skill in the art that the use of the singular includes the plural unless specifically stated otherwise. Therefore, the terms “a”, “an” and “at least one” are used interchangeably in this application.

[0112] For purposes of better understanding the present teachings and in no way limiting the scope of the teachings, unless otherwise indicated, all numbers expressing quantities, percentages or proportions, and other numerical values used in the specification and claims, are to be understood as being modified in all instances by the term “about.” Accordingly, unless indicated to the contrary, the numerical parameters set forth in the following specification and attached claims are approximations that may vary depending upon the

desired properties sought to be obtained. At the very least, each numerical parameter should at least be construed in light of the number of reported significant digits and by applying ordinary rounding techniques.

[0113] In the description and claims of the present application, each of the verbs, “comprise”, “include”, and “have” and conjugates thereof, are used to indicate that the object or objects of the verb are not necessarily a complete listing of components, elements or parts of the subject or subjects of the verb.

[0114] Other terms as used herein are meant to be defined by their well-known meanings in the art.

[0115] Additional objects, advantages, and novel features of the present invention will become apparent to one ordinarily skilled in the art upon examination of the following examples, which are not intended to be limiting. Additionally, each of the various embodiments and aspects of the present invention as delineated hereinabove and as claimed in the claims section below finds experimental support in the following examples.

[0116] It is appreciated that certain features of the invention, which are, for clarity, described in the context of separate embodiments, may also be provided in combination in a single embodiment. Conversely, various features of the invention, which are, for brevity, described in the context of a single embodiment, may also be provided separately or in any suitable sub-combination or as suitable in any other described embodiment of the invention. Certain features described in the context of various embodiments are not to be considered essential features of those embodiments, unless the embodiment is inoperative without those elements.

EXAMPLES

[0117] Generally, the nomenclature used herein, and the laboratory procedures utilized in the present invention include molecular, biochemical, microbiological and recombinant DNA techniques. Such techniques are thoroughly explained in the literature. See, for example, "Molecular Cloning: A laboratory Manual" Sambrook et al., (1989); "Current

Protocols in Molecular Biology" Volumes I-III Ausubel, R. M., ed. (1994); Ausubel et al., "Current Protocols in Molecular Biology", John Wiley and Sons, Baltimore, Maryland (1989); Perbal, "A Practical Guide to Molecular Cloning", John Wiley & Sons, New York (1988); Watson et al., "Recombinant DNA", Scientific American Books, New York; Birren et al. (eds.) "Genome Analysis: A Laboratory Manual Series", Vols. 1-4, Cold Spring Harbor Laboratory Press, New York (1998); methodologies as set forth in U.S. Pat. Nos. 4,666,828; 4,683,202; 4,801,531; 5,192,659 and 5,272,057; "Cell Biology: A Laboratory Handbook", Volumes I-III Cellis, J. E., ed. (1994); "Culture of Animal Cells - A Manual of Basic Technique" by Freshney, Wiley-Liss, N. Y. (1994), Third Edition; "Current Protocols in Immunology" Volumes I-III Coligan J. E., ed. (1994); Stites et al. (eds), "Basic and Clinical Immunology" (8th Edition), Appleton & Lange, Norwalk, CT (1994); Mishell and Shiigi (eds), "Strategies for Protein Purification and Characterization - A Laboratory Course Manual" CSHL Press (1996); all of which are incorporated by reference. Other general references are provided throughout this document.

Packaging of HA-Grin2Bwt within AAV_{BBB} and assessing function and expression in vitro

[0118] To assess whether AAVs can package, and subsequently deliver, *Grin2B* to mammalian cells, the inventors first tagged the subunit with an HA-epitope for specific immuno-detection of the subunit. This strategy allowed to differentiate the ectopic subunit from the endogenous ones. The inventors confirmed that tagging of the subunit had no effect over the receptor's expression, pharmacology and function, as assessed by two-electrode voltage clamp (TEVC) electrophysiology of *Xenopus* oocytes co-injected with mRNAs of *Grin1a* and HA-*Grin2Bwt* (**Figs. 1-2**). The inventors then cloned HA-*Grin2Bwt* into an AAV suitable vector. To remain below packaging capacity, the inventors turned towards exploring various 'short' promoters (the length of the gene cannot be altered). The inventors produced three clones under neuron-selective promoters, specifically hSYN promoter (448 bps), mini-promoter *pCALM1* (denoted mp-120, 120 bps) and the mini-promoter mp-84 (84 bps) (**Fig. 3**), and examined expression (by immunolabeling and electrophysiology) of the transgene in cultured mammalian cells (HEK293t) co-expressed

with the essential *Grin1a*. Of note, although the hSYN promoter is neuron-selective, it can drive moderate expression in HEK293t cells—but only via DNA-transfection (not infection), compared to ubiquitous promoters such as CMV. All three promoters gave rise to membrane expression of functional HA-GluN2B-containing receptors (**Fig. 4A**), compared to non-tagged *human-* or *rat* GluN2B-containing receptors (**Figs. 4B-4D**). Specifically, hSYN and mp-120 yielded comparable expression levels (**Fig. 4D**), whereas mp-84 required an internal intron (~100 bps, lengthening its sequence to 184 bps) for expression of YFP or HA-GluN2B (**Fig. 4C, 4D**). The inventors confirmed these observations in cultured neurons by means of DNA-transfection (**Fig. 5**). The inventors noted that hSYN provided a more pronounced expression. This is important as it provides the means to control levels of expression of the protein. Importantly, the inventors detected expression of the subunit at dendrites and synapses (dendritic spines), where the endogenous subunits are known to reside. Thus, HA-GluN2B is functional and expressed efficiently via three different short promoters in neurons, at the expected synaptic loci. Importantly, the inventors see no signs of excitotoxicity. The inventors then examined whether HA-*Grin2Bwt* could be packaged and expressed in cultured neurons following AAV-infection. Of note, all AAVs (regardless of serotype) contain an identical packaging capacity which led the inventors to initially attempt to package HA-*Grin2B* within AAV1. Surprisingly, the inventors obtained very high titers with the hSYN promoter (10^{12} particles/ml), suggesting viable particles. The inventors confirmed viability of viruses by infecting primary cultured neurons (**Fig. 6**) and exhibited that hSYN promoter drives the highest expression of HA-rNR2B amongst the tested promoters as verified by western blot (**Figs. 7-9**). These successes were quickly followed by production of a BBB-permeable variant, denoted bCAP10 and its injection into the tail vein of naïve (non-transgenic) C57/bl mice. Indeed, the inventors observed expression in many cortical neurons following a single injection (**Fig. 10**). Collectively, these results demonstrate that the *Grin2Bwt* gene can be packaged within AAVs, to yield high and suitable titers of functional (e.g., infectious) viruses, and that the subunit can be expressed in neurons of the brain via AAV_{BBB}. These strongly support the feasibility of the current invention.

Assessing functional and behavioral rescue following introduction of the Grin2Bwt gene into two unique animal models

[0119] The inventors aim at utilizing two unique *Grin2B*-animal models. The first is a heterozygous knock-out rat model missing one copy of *Grin2B*. This model is representative of haploinsufficiency cases. The partial behavioral phenotype of this model has been recently described, showing a higher incidence of spontaneous absence seizures (though mild), as in *GRIN*-patients. Currently, the inventors are further exploring the behavioral phenotype of this model (**Fig. 12**).

[0120] The second animal model is a unique *Grin2B*-knock-in mouse model (*Grin2B* c.2065G>T, translating into GluN2B-G689C, denoted $Grin2B^{+/G689C}$) to mimic the genotype of patients. In this model, the inventors are exploring rescue of electrophysiological and behavioral aspects by introduction of *Grin2Bwt* on top of the mixed genotype of the animals. Preliminary recordings show no deficits in neuronal firing features (**Fig. 13**), however, some deficits in NMDAR-currents (spontaneous EPSCs) in $Grin2B^{+/G689C}$- neurons compared to $Grin2B^{+/+}$ siblings appear (**Fig. 14**). Deficits in long term potentiation in $Grin2B^{+/G689C}$ female mice were observed (**Fig. 15**). Additionally, the inventors have begun exploring behavior of these animals using Morris Water Maze, Activity cages (smart-cages), rotarod, and open field (**Fig. 16**).

[0121] Lastly, after successfully packaging the largest *GRIN* gene (*Grin2B*; ~4.5 Kbp) into AAVs, the inventors now expanded their scope and packed *Grin1a* (2.9 Kbp) and *Grin2D* (4 Kbp) into AAVs and expressed them in neurons (**Fig. 17**). To do so the inventors tagged *Grin1a* and *Grin2D* with the HA epitope and cloned them under the hSYN promoter into a suitable vector for producing viral particles.

[0122] While certain features of the invention have been illustrated and described herein, many modifications, substitutions, changes, and equivalents will now occur to those of ordinary skill in the art. It is, therefore, to be understood that the appended claims are intended to cover all such modifications and changes as fall within the true spirit of the invention.

CLAIMS

What is claimed is:

1. A recombinant adeno-associated virus (AAV) vector comprising:

(a) a nucleic acid sequence encoding an N-methyl-D-aspartate (NMDA) receptor subunit; and

(b) a promoter having a length ranging between 50 and 500 bp,

wherein said nucleic acid sequence and said promoter are operably linked.

2. The recombinant AAV vector of claim 1, wherein a length of said nucleic acid sequence and of said promoter combined, ranges between 4 Kilobase pairs (Kbp) and 5 Kbp.

3. The recombinant AAV vector of claim 1 or 2, wherein a length of said nucleic acid sequence ranges between 4 Kbp and 5 Kbp.

4. The recombinant AAV vector of any one of claims 1 to 3, wherein said nucleic acid sequence comprises a sequence encoding a wildtype (w.t.) NMDA receptor subunit.

5. The recombinant AAV vector of any one of claims 1 to 4, wherein said promoter is a neuron-specific promoter.

6. The recombinant AAV vector of any one of claims 1 to 5, wherein said NMDA receptor subunit is selected from the group consisting of: GluN2B, GluN1, GluN2A, GluN2C, GluN2D, GluN3A, and GluN3B.

7. The recombinant AAV vector of any one of claims 1 to 6, wherein said promoter is selected from the group consisting of: mini-promoter mp-84, human synapsin (hSYN) promoter, and mini-promoter pCALM1 (mp-120).

8. The recombinant AAV vector of any one of claims 1 to 7, further comprising a post-transcriptional regulatory element.

9. The recombinant AAV vector of claim 8, wherein said post-transcriptional regulatory element comprises a woodchuck hepatitis virus post-transcriptional regulatory element (WPRE).

10. A pharmaceutical composition comprising a therapeutically effective amount of the recombinant AAV vector of any one of claims 1 to 9, and a pharmaceutically acceptable carrier.

11. The pharmaceutical composition of claim 10, for use in the treatment of an NMDA receptor-related encephalopathy in a subject in need thereof.

12. The pharmaceutical composition of claim 11, wherein said NMDA receptor-related encephalopathy is selected from the group consisting of: GRIN1-related neurodevelopmental disorder, GRIN2A developmental disorder, GRIN2B epilepsy, GRIN2A-related epilepsy-aphasia spectrum disorders, GRIN2B-related neurodevelopmental disorder, GRIN2D-related epileptic encephalopathy, Anti-NMDA receptor encephalitis, GRIN2C-associated intellectual disability, GRIN3A-related neurodevelopmental disorder, GRIN3B-associated neurological conditions, GRIN2A-related speech disorders, GRIN1-related epileptic encephalopathy, GRIN2B-associated autism spectrum disorder, GRIN2D-related movement disorders, GRIN1-associated schizophrenia, GRIN2A-related Landau-Kleffner syndrome, and GRIN2B-related West syndrome.

13. A method for treating an NMDA receptor-related encephalopathy in a subject in need thereof, the method comprising administering to the subject a therapeutically effective amount of a recombinant AAV vector comprising: (a) a nucleic acid sequence encoding an NMDA receptor subunit; and (b) a promoter having a length ranging between 50 and 500 bp, wherein said nucleic acid sequence and said promoter are operably linked.

14. The method of claim 13, wherein said NMDA receptor-related encephalopathy is selected from the group consisting of: GRIN1-related neurodevelopmental disorder, GRIN2A developmental disorder, GRIN2B epilepsy, GRIN2A-related epilepsy-aphasia spectrum disorders, GRIN2B-related neurodevelopmental disorder, GRIN2D-related

epileptic encephalopathy, Anti-NMDA receptor encephalitis, GRIN2C-associated intellectual disability, GRIN3A-related neurodevelopmental disorder, GRIN3B-associated neurological conditions, GRIN2A-related speech disorders, GRIN1-related epileptic encephalopathy, GRIN2B-associated autism spectrum disorder, GRIN2D-related movement disorders, GRIN1-associated schizophrenia, GRIN2A-related Landau-Kleffner syndrome, and GRIN2B-related West syndrome.

15. The method of claim 13 or 14, wherein said administering comprises intravenously administering, intranasally administering, cerebrospinal fluid (CFS) administering, intracerebroventricularly administering, or any combination thereof.

16. The method of any one of claims 13 to 15, wherein said recombinant AAV vector is in a pharmaceutical, and wherein said pharmaceutical composition further comprises a pharmaceutically acceptable carrier, a blood-brain barrier (BBB) permeability enhancing agent, or both.

17. The method of any one of claims 13 to 16, wherein a length of said nucleic acid sequence and of said promoter combined in said recombinant AAV vector, ranges between 4 Kilobase pairs (Kbp) and 5 Kbp.

18. The method of any one of claims 13 to 17, wherein a length of said nucleic acid sequence ranges between 4 Kbp and 5 Kbp.

19. The method of any one of claims 13 to 18, wherein said nucleic acid sequence comprises a sequence encoding a w.t. NMDA receptor subunit.

20. The method of any one of claims 13 to 19, wherein said promoter is a neuron-specific promoter.

21. The method of any one of claims 13 to 20, wherein said NMDA receptor subunit is selected from the group consisting of: GluN2B, GluN1, GluN2A, GluN2C, GluN2D, GluN3A, and GluN3B.

22. The method of any one of claims 13 to 21, wherein said promoter is selected from the group consisting of: mini-promoter mp-84, hSYN promoter, and mini-promoter pCALM1 (mp-120).

ABSTRACT

The present invention is directed to a recombinant adeno-associated virus (AAV) vector including: (a) a nucleic acid sequence encoding an N-methyl-D-aspartate (NMDA) receptor subunit; and (b) a promoter having a length ranging between 50 and 500 bp. The present invention is further directed to a method for treating N-methyl-D-aspartate (NMDA) receptor-related encephalopathy in a subject in need thereof, including administering to the subject a therapeutically effective amount of the recombinant AAV vector of the invention.