SUMMARY

The disclosure provides materials and methods useful for control of organ function to prevent, inhibit or treat disease. In one example, the method provides for delivery of viral vectors to organs which are then taken up by the axons of nerves which regulate function of those organs. In one example, the viral vector is an adeno-associated virus (AAV) vector. In one example, a retrograde form of adeno-associated virus (AAV), when injected into the wall of the stomach, is specifically taken up into a subset of vagus nerve sensory neurons which respond to distention of the stomach and cause satiety. 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. This represents a specific subset of neurons emanating from the nodose ganglion, while not affecting other nodose neurons which provide sensation to other visceral organs. The viral vector can also be other forms of retrograde vectors, including but not limited to retrograde lentiviral (LV) vectors, herpes simplex virus (HSV) vectors or canine adenovirus (CAV) vectors.

In one aspect, the present disclosure refers to use of a viral vector in the manufacture of a medicament for preventing, inhibiting or treating a cough in a mammal, wherein the mammal has a parasympathetic nerve fiber that innervates the lung of the mammal; the viral vector expresses a gene encoding a designer receptor exclusively activated by designer drugs (DREADD), wherein the DREADD inhibits neuronal activity in the presence of an agent that binds to the DREADD~~protein, the activity of which is inhibited by an agent or energy~~; and the parasympathetic nerve fiber is to be infected with the viral vector.

In another aspect, the present disclosure refers to use of a viral vector in the manufacture of a first medicament and use of an agent ~~or energy~~ in the manufacture of a second medicament, wherein the first and second medicaments are to be used in combination therapy to prevent, inhibit or treat a cough in a mammal, wherein the mammal has a parasympathetic nerve fiber that innervates the lung of the mammal; the viral vector expresses a gene encoding a DREADD~~protein~~, wherein the DREADD inhibits neuronal activity in the presence of an agent that binds to the DREADD~~the activity of which is inhibited by the agent or energy~~; the parasympathetic nerve fiber is to be infected with the viral vector; and the agent is to be administered ~~or the energy is to be delivered~~ to the mammal in an amount effective to inhibit the activity of the protein.

In one example, a method is provided to deliver one or more genes to nerve fibers that control function of an organ, e.g., a visceral organ including but not limited to stomach, small intestine, large intestine, pancreas, liver, spleen, gall bladder, lung, kidney, and heart. In one example, a viral gene therapy vector is delivered into a region of an organ that is innervated by a regulatory nerve such as a vagus nerve, cardiopulmonary nerve, thoracic splanchnic nerve, lumbar splanchnic nerve, sacral splanchnic nerve or pelvic splanchnic nerve. In one example, the organ is a stomach, intestines, pancreas, liver, lung, heart, adrenal, kidney, gonads, bladder, anal sphincter or urinary sphincter. In one example the viral vector is modified for retrograde transport in the central nervous system. In one example, the viral vector is injected into the organ. In one example, the delivery of the vector prevents, inhibits or treats a disease. In one example, the viral vector is injected into the stomach and the expression of the gene controls food intake. In one example, the viral vector is delivered to the lung, e.g., via inhalation, and the expression of the gene controls cough. In one example, expression of the gene activates the regulatory nerve. In one example, expression of the gene inhibits the regulatory nerve.

~~In another aspect, the present disclosure refers to use of a viral vector in the manufacture of a medicament for controlling visceral organ function in a mammal; wherein the mammal has a regulatory nerve which innervates the visceral organ of the mammal; the viral vector comprises a gene encoding a gene product, the activity of which protein is inhibited or activated by administration of an agent or delivery of an energy; and the regulatory nerve is to be infected with the viral vector.~~

~~In another aspect, the present disclosure refers to use of a viral vector in the manufacture of a first medicament and use of an agent or energy in the manufacture of a second medicament, wherein the first and second medicaments are to be used in combination therapy to control visceral organ function in a mammal; wherein the mammal has a regulatory nerve which innervates the visceral organ of the mammal; the viral vector comprises a gene encoding a gene product, the activity of which protein is inhibited or activated by administration of an agent or delivery of an energy; the regulatory nerve is to be infected with the viral vector; and the agent is to be administered or the energy is to be delivered in an amount effective to control the visceral organ function.~~

The disclosure also provides for a viral vector with improved properties for retrograde uptake into target neurons. This vector contains a capsid, which contains a mix of capsid proteins from one serotype of AAV, e.g., AAV serotype 2, with point mutations increasing retrograde uptake (retroAAV) (Tevro, et al., Neuron 92:372-378 (2016), which is incorporated by reference herein) and capsid proteins from a different serotype of AAV, e.g., AAV serotype rh10. This capsid mix creates a viral vector that has dramatically enhanced retrograde uptake and efficiency into afferent neurons compared with capsids that contain protein exclusively from retroAAV. This vector provides for efficient control of organ function.

~~In another aspect, the present disclosure refers to a recombinant AAV (rAAV) comprising a capsid formed of capsid proteins from two or more different AAV capsid serotypes.~~

The disclosure also provides a method for regulated control of organ function. The method generally includes delivery of a gene via a retrograde vector to neurons afferent to the organs, the product of which responds to an external drug or stimulus to control organ function. In one example, this method can be used to induce satiety and reduce food intake in order to control body weight. In this example, retrograde AAV (retroAAV) expressing a Designer Receptor Exclusively Activated by Designer Drugs (DREADD) that activates neurons, is injected into the wall of the stomach and is taken up into vagus sensory neurons, afferent to the stomach, which respond to stomach stretch and induce satiety. Following systemic administration of a DREADD activator, e.g., clozapine-N-oxide (CNO), satiety is induced and food intake is decreased. Other examples include delivery of an excitatory chemogenetic ion channel to these neurons followed by activation with the appropriate drug or delivery of the excitatory optogenetic ion channel ChR2 followed by light delivery to the nerve fibers to activate ChR2. In another example, this method is used to control intractable cough that is not due to an otherwise treatable disease. In one example, retrograde AAV expressing an inhibitory DREADD is aerosolized and inhaled for uptake into vagus sensory neurons of the lung, followed by systemic administration of a DREADD activator, e.g., CNO, to inhibit activity of these sensory neurons to reduce the cough reflex. In one example, retrograde AAV expressing, for instance, an inhibitory DREADD, is injected, e.g., into the vagus verve or nodose ganglion, followed by systemic administration of a DREADD activator, e.g., CNO, to inhibit activity of these sensory neurons to reduce the cough reflex.

In one example, the viral vector encodes hM4Di, an engineered version of the M4 muscarinic acetylcholine receptor which, when bound by CNO, clozapine, perlapine, or compound 21 (see Chen et al., ACS Chem. Neurosic., 6:476 (2015)),

WHAT IS CLAIMED IS:

1. Use of a viral vector in the manufacture of a medicament for preventing, inhibiting or treating a cough in a mammal, wherein the mammal has a parasympathetic nerve fiber that innervates the lung of the mammal; the viral vector expresses a gene encoding a designer receptor exclusively activated by designer drugs (DREADD), wherein the DREADD inhibits neuronal activity in the presence of an agent that binds to the DREADD~~protein, the activity of which is inhibited by an agent or energy~~; and the parasympathetic nerve fiber is to be infected with the viral vector.

2. The use of claim 1, wherein the viral vector is to be administered to the mammal via inhalation or injection, or intravenously.

3. The use of claim 1 or 2, wherein the agent is to be administered ~~or the energy is to be delivered~~ to the mammal in an amount effective to inhibit the activity of the DREADD~~protein~~.

4. The use of ~~claim 1, 2 or 3~~any one of claims 1 to 3, wherein the mammal is a human.

5. The use of any one of claims 1 to 4, wherein the mammal has idiopathic cough or intractable cough.

6. The use of any one of claims 1 to 4, wherein the mammal has chronic obstructive pulmonary disease (COPD) or gastric reflux.

7. The use of any one of claims 1 to 6, wherein the viral vector is an adeno-associated virus, lentivirus, adenovirus, or herpes simplex virus vector.

8. The use of any one of claims 1 to 7, wherein the viral vector is a retrograde form of adeno-associated virus, lentivirus or canine adenovirus.

9. The use of any one of claims 1 to 8, wherein the viral vector is modified for retrograde transport.

10. The use of any one of claims 1 to 9, wherein the DREADD is an engineered M4 muscarinic acetylcholine receptor~~gene encodes a light-sensitive ion channel (optogenetics), a chemically-responsive ion channel (chemogenetics), an ultrasound-sensitive ion channel (sonogenetics), a magnetic-field responsive ion-channel (magnetogenetics) or a designer receptor exclusively activated by designer drugs (DREADDs)~~.

11. The use of claim 10, wherein the DREADD is a hM4Di receptor.

~~11~~12. The use of any one of claims 1 to ~~10~~11, wherein the viral vector is a rAAV comprising an adeno-associated viral capsid comprising two or more different AAV capsid serotypes.

~~12~~13. The use of ~~any one of claims 6 to 11~~claim 12, wherein the rAAV comprises capsid proteins from a retro-AAV capsid serotype~~one of the AAV serotypes comprises AAV2~~.

~~13~~14. The use of any one of claims ~~6 to 12~~12 or 13, wherein the rAAV comprises capsid proteins from a~~one of the AAV serotypes comprises~~ AAVrh10 capsid serotype.

15. The use of claim 14, wherein the rAAV comprises capsid proteins from a retro-AAV capsid serotype and a AAVrh10 capsid serotype.

16. The use of any one of claims 1 to 15, wherein the agent that binds to the DREADD is clozapine-N-oxide (CNO).

~~14. Use of a viral vector in the manufacture of a medicament for controlling visceral organ function in a mammal; wherein the mammal has a regulatory nerve which innervates the visceral organ of the mammal; the viral vector comprises a gene encoding a gene product, the activity of which protein is~~

~~inhibited or activated by administration of an agent or delivery of an energy; and the regulatory nerve is to be infected with the viral vector.~~

~~15. The use of claim 14, wherein the nerve fiber is the vagus nerve, cardiopulmonary nerve, thoracic splanchnic nerve, lumbar splanchnic nerve, sacral splanchnic nerve or pelvic splanchnic nerve or the mammalian organ is a stomach, intestine, pancreas, liver, lung, heart, adrenal, kidney, gonad, or bladder.~~

~~16. The use of claim 14 or 15, wherein the agent is to be administered or the energy is to be delivered to the mammal in an amount effective to inhibit the activity of the protein.~~

~~17. The use of any one of claims 14 to 16, wherein the viral vector is an adeno-associated virus, lentivirus, adenovirus, or herpes simplex virus vector.~~

~~18. The use of any one of claims 14 to 17, wherein the viral vector provides for retrograde transport in neurons.~~

~~19. The use of any one of claims 14 to 17, wherein the viral vector is modified to provide for retrograde transport in the central nervous system.~~

~~20. The use of any one of claims 14 to 17, wherein the viral vector is a retrograde form of adeno-associated virus, lentivirus or canine adenovirus.~~

~~21. The use of any one of claims 14 to 20, wherein the gene product regulates the activity of the nerve controlling the organ in response to the administration of the agent.~~

~~22. The use of any one of claims 14 to 21, wherein the gene encodes a light-sensitive ion channel, a chemically-responsive ion channel, an ultrasound-sensitive ion channel, a magnetic-field responsive ion channel or a designer receptor exclusively activated by designer drugs (DREADDs).~~

~~23. The use of claim 22 wherein the gene encodes a channelrhodopsin, nicotinic acetylcholine receptor, gramicidin A, a voltage-gated potassium channel, an ionotropic glutamate receptor, alpha-hemolysin, or a mechanosensitive channel.~~

~~24. The use of any one of claims 14 to 23 wherein the viral vector is to be delivered to the vagus nerve.~~

~~25. The use of any one of claims 14 to 24 wherein the agent administered or the energy delivered allows for control of food intake, control of anal sphincter or control of urinary sphincter in the mammal.~~

~~26. The use of claim 25, wherein the visceral organ is a stomach, duodenum or small intestine.~~

~~27. The use of any one of claims 14 to 26, wherein the gene product blocks toxic protein spread to the vagus nerve of the mammal.~~

~~28. The use of any one of claims 25 to 27 wherein the viral vector is taken up retrograde from the gastrointestinal system.~~

~~29. The use of claim 27 or 28, wherein the toxic protein comprises alpha-synuclein, tau, beta-amyloid, or huntingtin.~~

~~30. The use of any one of claims 14 to 29, wherein the gene product comprises or encodes small hairpin RNA (shRNA), microRNA (miRNA), CrispR/Cas9, an antibody, a single-chain antibody, or an intrabody.~~

~~31. The use of any one of claims 27 to 30, wherein the amount administered or delivered prevents or inhibits spread of a toxic protein from the gastrointestinal tract to the brain in a mammal.~~

~~32. The use of any one of claims 14 to 31, wherein the mammal is a human.~~

~~33. The use of any one of claims 17 to 32, wherein one of the AAV serotypes comprises AAV2.~~

~~34. The use of any one of claims 17 to 33, wherein one of the AAV serotypes comprises AAVrh10.~~

~~35. The use of any one of claims 17 to 34 wherein the viral vector is a rAAV comprising an adeno-associated viral capsid comprising two or more different AAV capsid serotypes.~~

~~36. The use of any one of claims 14 to 35 wherein the amount administered prevents, inhibits or treats disease in a visceral organ in the mammal.~~

~~37~~17. Use of a viral vector in the manufacture of a first medicament and use of an agent ~~or energy~~ in the manufacture of a second medicament, wherein the first and second medicaments are to be used in combination therapy to prevent, inhibit or treat a cough in a mammal, wherein the mammal has a parasympathetic nerve fiber that innervates the lung of the mammal; the viral vector expresses a gene encoding a ~~protein~~DREADD, wherein the DREADD inhibits neuronal activity in the presence of an agent that binds to the DREADD~~the activity of which is inhibited by the agent or energy~~; the parasympathetic nerve fiber is to be infected with the viral vector; and the agent is to be administered ~~or the energy is to be delivered~~ to the mammal in an amount effective to inhibit the activity of the protein.

~~38~~18. The use of claim ~~37~~17, wherein the viral vector is to be administered to the mammal via inhalation or injection.

~~39~~19. The use of claim ~~37~~ 17 or ~~38~~18, wherein the agent is to be intravenously administered.

~~40~~20. The use of any one of claims ~~37, 38 or 39~~17 to 19, wherein the mammal is a human.

~~41~~21. The use of any one of claims ~~37~~ 17 to ~~40~~20, wherein the mammal has idiopathic cough or intractable cough.

~~42~~22. The use of any one of claims ~~37~~ 17 to ~~40~~20, wherein the mammal has chronic obstructive pulmonary disease (COPD) or gastric reflux.

~~43~~23. The use of any one of claims ~~37~~ 17 to ~~42~~22, wherein the viral vector is an adeno-associated virus, lentivirus, adenovirus, or herpes simplex virus vector.

~~44~~24. The use of any one of claims ~~37~~ 17 to ~~43~~23, wherein the viral vector is a retrograde form of adeno-associated virus, lentivirus or canine adenovirus.

~~45~~25. The use of any one of claims ~~37~~ 17 to ~~44~~24, wherein the viral vector is modified for retrograde transport.

~~46~~26. The use of any one of claims ~~37~~ 17 to ~~45~~25, wherein the DREADD is an engineered M4 muscarinic acetylcholine receptor~~the gene encodes a light-sensitive ion channel (optogenetics), a chemically-responsive ion channel (chemogenetics), an ultrasound-sensitive ion channel (sonogenetics), a magnetic-field-responsive ion channel (magnetogenetics) or a designer receptor exclusively activated by designer drugs (DREADDs)~~.

27. The use of claim 26, wherein the DREADD is a hM4Di receptor.

~~47~~28. The use of any one of claims ~~37~~ 17 to ~~46~~27, wherein the viral vector is a rAAV comprising an adeno-associated viral capsid comprising two or more different AAV capsid serotypes.

~~48~~29. The use of ~~any one of claims 42 to 47~~claim 28, wherein the rAAV comprises capsid proteins from a retro-AAV capsid serotype~~wherein one of the AAV serotypes comprises AAV2~~.

~~49~~30. The use of ~~any one of claims 42 to 48~~claim 28 or 29, wherein the rAAV comprises capsid proteins from a AAVrh10 capsid serotype~~wherein one of the AAV serotypes comprises AAVrh10~~.

31. The use of claim 30, wherein the rAAV comprises capsid proteins from a retro-AAV capsid serotype and a AAVrh10 capsid serotype.

32. The use of any one of claims 17 to 31, wherein the agent that binds to the DREADD is CNO.

~~50. Use of a viral vector in the manufacture of a first medicament and use of an agent or energy in the manufacture of a second medicament, wherein the first and second medicaments are to be used in combination therapy to control visceral organ function in a mammal; wherein the mammal has a regulatory nerve which innervates the visceral organ of the mammal; the viral vector comprises a gene encoding a gene product, the activity of which protein is inhibited or activated by administration of an agent or delivery of an energy; the regulatory nerve is to be infected with the viral vector; and the agent is to be administered or the energy is to be delivered in an amount effective to control the visceral organ function.~~

~~51. The use of claim 50, wherein the nerve fiber is the vagus nerve, cardiopulmonary nerve, thoracic splanchnic nerve, lumbar splanchnic nerve, sacral splanchnic nerve or pelvic splanchnic nerve or the mammalian organ is a stomach, intestine, pancreas, liver, lung, heart, adrenal, kidney, gonad, or bladder.~~

~~52. The use of claim 50 or 51, wherein the agent is to be administered or the energy is to be delivered to the mammal in an amount effective to inhibit the activity of the protein.~~

~~53. The use of any one of claims 50 to 52, wherein the viral vector is an adeno-associated virus, lentivirus, adenovirus, or herpes simplex virus vector.~~

~~54. The use of any one of claims 50 to 53, wherein the viral vector provides for retrograde transport in neurons.~~

~~55. The use of any one of claims 50 to 53, wherein the viral vector is modified to provide for retrograde transport in the central nervous system.~~

~~56. The use of any one of claims 50 to 53, wherein the viral vector is a retrograde form of adeno-associated virus, lentivirus or canine adenovirus.~~

~~57. The use of any one of claims 50 to 56, wherein the gene product regulates the activity of the nerve controlling the organ in response to the administration of the agent.~~

~~58. The use of any one of claims 50 to 57, wherein the gene encodes a light-sensitive ion channel, a chemically-responsive ion channel, an ultrasound-sensitive ion channel, a magnetic-field responsive ion channel or a designer receptor exclusively activated by designer drugs (DREADDs).~~

~~59. The use of claim 58 wherein the gene encodes a channelrhodopsin, nicotinic acetylcholine receptor, gramicidin A, a voltage-gated potassium channel, an ionotropic glutamate receptor, alpha-hemolysin, or a mechanosensitive channel.~~

~~60. The use of any one of claims 50 to 59 wherein the viral vector is to be delivered to the vagus nerve.~~

~~61. The use of any one of claims 50 to 60 wherein the agent administered or the energy delivered allows for control of food intake, control of anal sphincter or control of urinary sphincter in the mammal.~~

~~62. The use of claim 61, wherein the visceral organ is a stomach, duodenum or small intestine.~~

63. The use of any one of claims 50 to 62, wherein the gene product blocks toxic protein spread to the vagus nerve of the mammal.

64. The use of any one of claims 61 to 63 wherein the viral vector is taken up retrograde from the gastrointestinal system.

65. The use of claim 63 or 64, wherein the toxic protein comprises alpha-synuclein, tau, beta-amyloid, or huntingtin.

66. The use of any one of claims 50 to 65, wherein the gene product comprises or encodes small hairpin RNA (shRNA), microRNA (miRNA), CrispR/Cas9, an antibody, a single-chain antibody, or an intrabody.

67. The use of any one of claims 63 to 66, wherein the amount administered or delivered prevents or inhibits spread of a toxic protein from the gastrointestinal tract to the brain in a mammal.

68. The use of any one of claims 50 to 67, wherein the mammal is a human.

69. The use of any one of claims 53 to 68, wherein one of the AAV serotypes comprises AAV2.

70. The use of any one of claims 53 to 69, wherein one of the AAV serotypes comprises AAVrh10.

71. The use of any one of claims 53 to 70 wherein the viral vector is a rAAV comprising an adeno-associated viral capsid comprising two or more different AAV capsid serotypes.

72. The use of any one of claims 50 to 71 wherein the amount administered prevents, inhibits or treats disease in a visceral organ in the mammal.

73. A recombinant AAV (rAAV) comprising a capsid formed of capsid proteins from two or more different AAV capsid serotypes.

~~74. The recombinant AAV of claim 73, wherein one of the serotypes comprises AAV2.~~

~~75. The recombinant AAV of claim 73 or 74, wherein one of the serotypes comprises AAVrh10.~~

~~76. The recombinant AAV of claim 73, 74 or 75 which provides for retrograde delivery.~~

~~77. The recombinant AAV of any one of claims 73 to 76, wherein the rAAV encodes a therapeutic gene product, a prophylactic gene product or an exogenously controllable protein.~~

~~78. The recombinant AAV of claim 77 which encodes a light-sensitive ion channel (optogenetics), a chemically-responsive ion channel (chemogenetics), an ultrasound-sensitive ion channel (sonogenetics), a magnetic-field responsive ion channel (magnetogenetics) or a designer receptor exclusively activated by designer drugs (DREADDs).~~

~~79. The recombinant AAV of claim 77 or 78 which encodes a channel protein or DREADD that is inhibited by exogenous administration of an agent or energy.~~

~~80. The recombinant AAV of claim 79 which encodes hD3q.~~

~~81. The recombinant AAV of any one of claims 73 to 80 wherein the capsid comprises a capsid having at least 80%, 85%, 90%, 92%, 95%, 96%, 97%, 98% or 99% amino acid sequence identity to SEQ ID NO:5 or 7.~~

~~82. The rAAV of claim 76, wherein the rAAV provides for retrograde transport in the central nervous system.~~

~~83. The rAAV of claim 77, wherein the gene product regulates the activity of a parasympathetic nerve fiber innervating a visceral organ in response to the administration of an agent.~~

~~84. The rAAV of claim 77, wherein the gene product is a channelrhodopsin, nicotinic a cetylcholine receptor, gramicidin A, a voltage-gated potassium channel, an ionotropic glutamate receptor, alpha-hemolysin, or a mechanosensitive channel.~~

~~85. The rAAV of claim 77, wherein the gene product blocks toxic protein spreads to the vagus nerve of the mammal.~~

~~86. The rAAV of claim 73, wherein rAAV is taken up retrograde from the gastrointestinal system.~~

~~87. The rAAV of claims 85 or 86, wherein the toxic protein comprises alpha-synuclein, tau, beta-amyloid, or huntingtin.~~

~~88. The rAAV of claim 77, wherein the gene product comprises or encodes small hairpin RNA (shRNA), microRNA (miRNA), CrispR/Cas9, an antibody, a single-chain antibody, or an intrabody.~~