

CLAIMS

What is claimed is:

1. Use of a nucleic acid comprising a nucleotide sequence encoding a medium wavelength opsin (MW-opsin) and/or a long wavelength opsin (LW-opsin) and/or a short wavelength opsin (SW-opsin) in the manufacture of a medicament for restoring or enhancing visual function in an individual having reduced visual function due to loss of photoreceptors, wherein said medicament provides for expression of the MW-opsin and/or the LW-opsin and/or the SW-opsin in a retinal cell in the individual and restoration or enhancement of visual function, wherein said nucleic acid does not comprise a nucleotide sequence encoding an opsin promoter and wherein said retinal cell is a ganglion cell, an amacrine cell, a horizontal cell or a bipolar cell.
2. The use of claim 1, wherein the MW-opsin comprises an amino acid sequence having at least 85% amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:1; wherein the LW-opsin comprises an amino acid sequence having at least 85% amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:4, and wherein the SW-opsin comprises an amino acid sequence having at least 85% amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:5.
3. The use of claim 1, wherein the SW-opsin comprises an amino acid sequence having at least 87% amino acid sequence identity to the human SW-opsin amino acid sequence set forth in SEQ ID NO:5 or having at least 87% amino acid sequence identity to the mouse SW-opsin amino acid sequence set forth in SEQ ID NO:6.
4. The use of claim 1, comprising administering to the individual: i) a first nucleic acid comprising a nucleotide sequence encoding an SW-opsin comprising an amino acid sequence having at least 87% amino acid sequence identity to the human SW-opsin amino acid sequence set forth in SEQ ID NO:5; and ii) a second nucleic acid comprising a nucleotide sequence encoding an SW-opsin comprising an amino acid sequence having at least 87% amino acid sequence identity to the mouse SW-opsin amino acid sequence set forth in SEQ ID NO:6.

5. The use of claim 1, wherein the SW-opsin is a chimeric SW-opsin comprising mouse SW-opsin intracellular domains and human SW-opsin transmembrane domains.

6. The use of claim 5, wherein the chimeric SW-opsin comprises an amino acid sequence having at least 90% amino acid sequence identity to the chimeric SW-opsin amino acid sequence set forth in SEQ ID NO:7.

7. The use of any one of claims 1-6, wherein expression of the MW-opsin and/or the LW-opsin and/or the SW-opsin in the retinal cell provides for patterned vision and image recognition by the individual.

8. The use of claim 7, wherein the image recognition is of a static image or a pattern.

9. The use of claim 7, wherein the image recognition is of a moving image or a pattern.

10. The use of any one of claims 1-9, wherein expression of the MW-opsin and/or the LW-opsin and/or the SW-opsin in the retinal cell provides for image recognition at a light intensity of from about 10^{-4} W/cm² to about 1 W/cm².

11. The use of any one of claims 1-9, wherein expression of the MW-opsin and/or the LW-opsin and/or the SW-opsin in the retinal cell provides for image recognition at a light intensity that is at least 10-fold lower than the light intensity required to provide for image recognition by an individual expressing a channelrhodopsin polypeptide in a retinal cell.

12. The use of any one of claims 1-9, wherein expression of the MW-opsin and/or the LW-opsin and/or the SW-opsin in the retinal cell provides for kinetics that are at least 2-fold faster than the kinetics conferred on a retinal cell by a rhodopsin polypeptide.

13. The use of any one of claims 1-12, wherein the nucleic acid is a recombinant expression vector.

14. The use of claim 13, wherein the recombinant expression vector is a recombinant viral vector.

15. The use of claim 14, wherein the recombinant viral vector is an adeno-associated viral vector, a lentiviral vector, a herpes simplex virus vector, or a retroviral vector.

16. The use of any one of claims 1-15, wherein the nucleotide sequence is operably linked to a transcriptional control element that is functional in a retinal cell.

17. The use of claim 16, wherein the transcriptional control element is a retinal cell-specific promoter.

18. The use of claim 17, wherein the promoter is a synapsin promoter, a CAG promoter, a CMV promoter, a grm6 promoter, a Pleiades promoter, a ChAT promoter, a V-glut promoter, a GAD promoter, a PV promoter, a somatostatin (SST) promoter, a neuropeptide Y (NPY) promoter, a VIP promoter, rhodopsin promoter, a rhodopsin kinase promoter, vitelliform macular dystrophy 2 (VMD2) gene promoter, or an interphotoreceptor retinoid-binding protein (IRBP) gene promoter.

19. The use of any one of claims 1-18, wherein said administering is via intraocular injection.

20. The use of any one of claims 1-18, wherein said administering is via intravitreal injection.

21. The use of any one of claims 1-18, wherein said administering is via subretinal injection.

22. The use of any one of claims 1-21, wherein the individual has an ocular disease selected from retinitis pigmentosa, macular degeneration, retinoschisis, and Leber's Congenital Amaurosis, and diabetic retinopathy.

23. The use of any one of claims 1-21, wherein the individual has experienced retinal detachment or photoreceptor loss due to trauma or head injury.

24. The use of any one of claims 1-23, wherein the nucleic acid is complexed with a nanoparticle.

25. A recombinant viral vector comprising a nucleotide sequence encoding a medium wavelength opsin (MW-opsin) and/or a long wavelength opsin (LW-opsin) and/or a short wavelength opsin (SW-opsin), wherein said vector does not comprise an opsin promoter.

26. The recombinant viral vector of claim 25, wherein the viral vector is an adeno-associated viral vector.

27. The recombinant viral vector of claim 25 or claim 26, wherein the MW-opsin comprises an amino acid sequence having at least 85% amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:1; wherein the LW-opsin comprises an amino acid sequence having at least 85% amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:4, and wherein the SW-opsin comprises an amino acid sequence having at least 87% amino acid sequence identity to the amino acid sequence set forth in one of SEQ ID NOs:5-7.

28. The recombinant viral vector of any one of claims 25-27, wherein the nucleotide sequence is operably linked to a promoter.

29. (The recombinant viral vector of claim 28, wherein the promoter is a synapsin promoter, a CAG promoter, a CMV promoter, a grm6 promoter, a Pleiades promoter, a ChAT promoter, a V-glut promoter, a GAD promoter, a PV promoter, a somatostatin (SST) promoter, a neuropeptide Y (NPY) promoter, a VIP promoter, rhodopsin promoter, a rhodopsin kinase promoter, vitelliform macular dystrophy 2 (VMD2) gene promoter, or an interphotoreceptor retinoid-binding protein (IRBP) gene promoter.

30. The recombinant viral vector of any one of claims 25-29, wherein the recombinant viral vector is a recombinant AAV vector, wherein the recombinant AAV vector

comprises a nucleotide sequence encoding a variant capsid polypeptide that confers increased infectivity of a retinal cell and/or confers increased ability to cross the inner limiting membrane, compared to a wild-type AAV capsid.

31. A pharmaceutical composition comprising:

- a) the recombinant viral vector of any one of claims 25-30; and
- b) a pharmaceutically acceptable excipient.

32. Use of the recombinant viral vector of any one of claims 25-29 in the manufacture of a medicament for restoring or enhancing visual function in an individual, wherein said medicament comprises the recombinant viral vector of any one of claims 25-30, or the pharmaceutical composition of claim 31, wherein administering the medicament to the individual provides for expression of the MW-opsin and/or the LW-opsin and/or the SW-opsin in a retinal cell in the individual and restoration or enhancement of visual function.

33. A chimeric SW-opsin comprising intracellular portions of a mouse SW-opsin comprising an amino acid sequence having at least 87% amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:6, and comprising transmembrane portions of a human SW-opsin comprising an amino acid sequence having at least 87% amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:5.

34. The chimeric SW-opsin of claim 33, comprising an amino acid sequence having at least 90% amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:7.

35. A recombinant expression vector comprising a nucleotide sequence encoding the chimeric SW-opsin of claim 33 or claim 34.

36. A composition comprising:

- a) the recombinant expression vector of claim 35; and
- b) a pharmaceutically acceptable excipient.

37. A composition comprising a pharmacologically acceptable carrier and one or more recombinant nucleic acid vectors comprising one or more nucleotide sequences encoding one or more cone opsins, wherein said nucleic acid vector does not comprise an opsin promoter, wherein, when said one or more nucleotide sequences is expressed in an eye of a subject in need thereof, said subject can distinguish between an image comprising a vertical line and an image comprising a horizontal line in a spatial pattern discrimination assay.

38. A composition comprising a pharmacologically acceptable carrier and one or more recombinant nucleic acid vectors comprising one or more nucleotide sequences encoding one or more cone opsins, wherein said nucleic acid vector does not comprise an opsin promoter, wherein, when said one or more nucleotide sequences is expressed in an eye of a subject in need thereof, said subject can distinguish between an image comprising a static line and an image comprising a moving line in a spatial pattern discrimination assay.

39. A composition comprising a pharmacologically acceptable carrier and one or more recombinant nucleic acid vectors comprising one or more nucleotide sequences encoding one or more cone opsins, wherein said nucleic acid vector does not comprise an opsin promoter, wherein, when said one or more nucleotide sequences is expressed in an eye of a subject in need thereof, said subject can distinguish between flashing light and constant light in a temporal light pattern assay.

40. A composition comprising a pharmacologically acceptable carrier and one or more recombinant nucleic acid vectors comprising one or more nucleotide sequences encoding one or more cone opsins, wherein said nucleic acid vector does not comprise an opsin promoter, wherein, when said one or more nucleotide sequences is expressed in an eye of a subject in need thereof, said subject can recognize an image at a light intensity of from about 10^{-4} W/cm² to about 10 W/cm² in an image recognition assay.

41. A composition comprising a pharmacologically acceptable carrier and one or more recombinant nucleic acid vectors comprising one or more nucleotide sequences encoding one or more cone opsins, wherein said nucleic acid vector does not comprise an opsin promoter, wherein, when said one or more nucleotide sequences is expressed in an eye of a subject in

need thereof, said subject can distinguish between an area with white light and an area without white light in a light avoidance assay.

42. The composition of any one of claims 37-41, wherein the one or more cone opsins is selected from the group consisting of:

a) an MW-opsin comprising an amino acid sequence having at least 85% amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:1;

b) an LW-opsin comprising an amino acid sequence having at least 85% amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:4;

c) an SW-opsin comprising an amino acid sequence having at least 85% amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:5; and

d) a chimeric SW-opsin comprising: i) intracellular portions of a mouse SW-opsin comprising an amino acid sequence having at least 87% amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:6; and ii) transmembrane portions of a human SW-opsin comprising an amino acid sequence having at least 87% amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:5.

43. The composition of any one of claims 37-41, wherein the one or more recombinant nucleic acid vectors comprise one or more nucleotide sequences encoding 2 different cone opsins.

44. The composition of any one of claims 37-41, wherein the one or more recombinant nucleic acid vectors comprise one or more nucleotide sequences encoding 3 different cone opsins.

45. The composition of any one of claims 37-44, wherein the one or more recombinant nucleic acid vectors are an adeno-associated viral vector, a lentiviral vector, a herpes simplex virus vector, or a retroviral vector.

46. The composition of any one of claims 37-44, wherein the one or more recombinant nucleic acid vectors are recombinant adeno-associated virus vectors.

47. The composition of claim 46, wherein the recombinant AAV vector comprises a nucleotide sequence encoding a variant capsid polypeptide that confers increased infectivity of a retinal cell and/or confers increased ability to cross the inner limiting membrane, compared to a wild-type AAV capsid.

48. The composition of any one of claims 37-47, wherein the one or more nucleotide sequences are operably linked to a promoter.

49. The composition of claim 48, wherein the promoter is a synapsin promoter, a CAG promoter, a CMV promoter, a grm6 promoter, a Pleiades promoter, a ChAT promoter, a V-glut promoter, a GAD promoter, a PV promoter, a somatostatin (SST) promoter, a neuropeptide Y (NPY) promoter, a VIP promoter, rhodopsin promoter, a rhodopsin kinase promoter, vitelliform macular dystrophy 2 (VMD2) gene promoter, or an interphotoreceptor retinoid-binding protein (IRBP) gene promoter.

50. The composition of any one of claims 37-49, wherein the subject has an ocular disease selected from retinitis pigmentosa, macular degeneration, retinoschisis, and Leber's Congenital Amaurosis, and diabetic retinopathy.

51. The composition of any one of claims 37-49, wherein the subject has experienced retinal detachment or photoreceptor loss due to trauma or head injury.

52. The composition of any one of claims 37-51, wherein the composition comprises a pharmaceutically acceptable excipient.

53. The composition of claim 52, wherein the pharmaceutically acceptable excipient comprises saline.

54. The composition of any one of claims 37-53, wherein the composition is sterile.