

THAT WHICH IS CLAIMED IS:

1. A method for producing a human striatal basal ganglia spheroids or organoids *in vitro*, the method comprising:

(a) inducing a human pluripotent stem cell in suspension culture to a neural fate to provide a neural spheroid;

(b) differentiating the neural spheroid into a striatal basal ganglia spheroid; and

(c) maintaining the striatal spheroid in neural medium, such that the striatal spheroid comprises human striatal neurons.

2. The method of claim 1, wherein the human striatal neurons comprise medium spiny neurons with mature morphology and electrophysiological properties.

3. The method of claim 2, wherein a mature morphology has neurons with spines.

4. The method of claim 2 or claim 3, wherein an electrophysiological property is inward rectification.

5. The method of any of claims 1-4, wherein the striatal spheroid further comprises at least one of the cells selected from the group consisting of: neural progenitor cells, radial glia and astrocytes.

6. The method of any of claims 1-5, wherein the human pluripotent stem cell is an induced human pluripotent stem cell.

7. The method of any of claims 1-6, wherein inducing the human pluripotent stem cell in suspension culture to the neural fate in step (a) comprises culturing in a medium comprising an inhibitor of bone morphogenetic protein (BMP) and an inhibitor of transforming growth factor β (TGF β), optionally wherein the medium further comprises an inhibitor of the Wnt pathway.

8. The method of claim 7, wherein the inhibitor of BMP is dorsomorphin and the inhibitor of TGF β is SB-431542.

9. The method of claim 7 or claim 8, wherein culturing in the medium comprising the inhibitor of BMP and inhibitor of TGF β is for a period of from 2 to 10 days.

10. The method of any of claims 1-9, wherein the suspension culture of step (a) is feeder layer free.

11. The method of any of claims 1-10, wherein differentiating the neural spheroid into the striatal spheroid of step (b) comprises culturing the neural spheroid in suspension culture in neural medium comprising an inhibitor of the Wnt pathway, an activin A, and a retinoid X receptor (RXR) agonist, optionally wherein the neural spheroid is cultured in neural medium comprising the inhibitor of the Wnt pathway and the activin A for a period of 4 to 8 weeks, and wherein the neural medium is supplemented with the RXR agonist after a period of 2 to 10 days.

12. The method of claim 11, wherein step (b) further comprises supplementing the neural medium with at least one of the compounds selected from the group consisting of: brain-derived neurotrophic factor (BDNF), NT3, L-Ascorbic Acid 2-phosphate Trisodium Salt (AA), N6, 2'-O-Dibutyryl adenosine 3', 5' -cyclic monophosphate sodium salt (cAMP), cis-4, 7, 10, 13, 16, 19- Docosahexaenoic acid (DHA), and DAPT, optionally wherein the neural medium is supplemented with the at least one compound after a period of 3 to 4 weeks.

13. The method of any of claims 1-12, wherein maintaining the striatal spheroid in step (c) is carried out in neural medium in the absence of growth factors.

14. The method of any of claims 1-13, wherein maintaining the striatal spheroid in step (c) is for at least 1 month.

15. The method of any of claims 1-14, wherein cells of the striatal spheroid comprise at least one allele associated with a neurologic or psychiatric disorder.

16. The method of claim 15, wherein the neurologic or psychiatric disorder is selected from the group consisting of: schizophrenia, obsessive-compulsive disorder, Tourette syndrome, Huntington's disease, Parkinson's disease and autism spectrum disorder (ASD).

17. A striatal spheroid comprising human striatal neurons, wherein the striatal spheroid is capable of being maintained in suspension culture without adhering to a surface for at least 1 month.

18. A striatal spheroid obtained by the method of any of claims 1-16.

19. A method for producing a cortico-striatal assembloid *in vitro*, the method comprising:

- (i) (a) inducing a first human pluripotent stem cell in suspension culture to a neural fate to provide a first neural spheroid;
- (b) differentiating the first neural spheroid into a striatal spheroid (hStrS),
- (ii)(a) inducing a second human pluripotent stem cell in a suspension culture to a neural fate to provide a second neural spheroid;
- (b) differentiating the second neural spheroid into a cortical spheroid (hCS), and
- (iii) culturing the hStrS and hCS under conditions permissive for cell fusion in neural medium, such that the cortico-striatal assembloid comprises human cortical neurons projecting from the hCS into the hStrS.

20. The method of claim 19, wherein the human cortical neurons comprise neurons expressing CTIP2 and/or neurons expressing SATB2, which can project and connect specifically with medium spiny neurons.

21. The method of claim 19 or claim 20, wherein the hStrS comprises human striatal neurons, optionally wherein the human striatal neurons comprise medium spiny neurons, optionally wherein the assembloid comprises a functional neural circuit between the human cortical neurons and human striatal neurons.

22. The method of any of claims 19-21 wherein fusion into a cortico-striatal assembloid induces functional maturation of medium spiny neurons.

23. The method of any of claims 19-22, wherein inducing the first and second human pluripotent stem cell in suspension culture to the neural fate in steps (i)(a) and (ii)(a) comprises culturing in a medium comprising an inhibitor of bone morphogenetic protein (BMP) and an inhibitor of transforming growth factor β (TGF β), optionally wherein the medium further comprises an inhibitor the Wnt pathway.

24. The method of claim 23, wherein the inhibitor of BMP is dorsomorphin and the inhibitor of TGF β is SB-431542.

25. The method of claim 23 or claim 24, wherein culturing in the medium comprising the inhibitor of BMP and inhibitor of TGF β is for a period of from 2 to 10 days.

26. The method of any of claims 19-25, wherein differentiating the first neural spheroid into the hStrS in step (i)(b) comprises culturing the first neural spheroid in suspension culture

in neural medium comprising an inhibitor of the Wnt pathway, an activin A, and a retinoid X receptor (RXR) agonist, optionally wherein the first neural spheroid is cultured in neural medium comprising the inhibitor of the Wnt pathway and the activin A agonist for a period of 4 to 8 weeks, and wherein the neural medium is supplemented with the RXR agonist after a period of 2 to 10 days.

27. The method of any of claims 19-26, wherein differentiating the second neural spheroid into the hCS in step (ii)(b) comprises:

culturing the second neural spheroid in suspension culture in medium comprising fibroblast growth factor 2 (FGF2) and epidermal growth factor (EGF) to provide cortical progenitor spheroids; and

culturing the cortical progenitor spheroids in suspension culture in medium lacking FGF2 and EGF and comprising brain-derived neurotrophic factor (BDNF) and neurotrophin 3 (NT3), L-Ascorbic Acid 2-phosphate Trisodium Salt (AA), N6, 2'-O-Dibutyryl adenosine 3', 5' -cyclic monophosphate sodium salt (cAMP), cis-4, 7, 10, 13, 16, 19- Docosahexaenoic acid (DHA).

28. The method of claim 27, wherein culturing the second neural spheroid in suspension culture in the medium comprising FGF2 and EGF is for a period of from 1 to 4 weeks, and culturing the neural progenitor spheroids in suspension culture in medium lacking FGF2 and EGF and comprising BDNF, NT3, AA, cAMP, DHA is for a period of from 1 to 4 weeks.

29. The method of any of claims 19-28, wherein step (iii) comprises culturing the hStrS and hCS are cultured under conditions permissive for cell fusion for at least 10 days.

30. The method of any of claims 19-29, wherein step (iii) comprises culturing the hStrS and hCS under conditions permissive for cell fusion comprises culturing the hStrS and hCS in close proximity to each other.

31. The method of any of claims 19-30, wherein the suspension culture of step (i)(a) and/or step (ii)(a) is feeder layer free.

32. The method of any of claims 19-31, wherein cells of the cortico-striatal assembloid comprise at least one allele associated with a neurologic or psychiatric disorder,

optionally wherein the neurologic or psychiatric disorder is selected from the group consisting of: schizophrenia, obsessive-compulsive disorder, Tourette syndrome, Huntington's disease, Parkinson's disease and autism spectrum disorder (ASD).

33. A cortico-striatal assembloid comprising a striatal spheroid (hStrS) fused to a cortical spheroid (hCS), wherein the cortico-striatal assembloid comprises human cortical neurons projecting from the hCS into the hStrS, and wherein the cortico-striatal assembloid is capable of being maintained in suspension culture without adhering to a surface for at least 4 weeks.

34. A cortico-striatal assembloid produced by the method of any of claims 19-32.

35. A method of determining the effect of a candidate agent on cortico-striatal neural circuits, the method comprising: contacting the candidate agent with the cortico-striatal assembloid of claim 33 or claim 34; and determining the effect of the candidate agent on the number and/or function of cortico-striatal neural circuits in the cortico-striatal assembloid,

optionally wherein cells of the cortico-striatal assembloid comprise at least one allele associated with a neurologic or psychiatric disorder, further optionally wherein the neurologic or psychiatric disorder is selected from the group consisting of: schizophrenia, obsessive-compulsive disorder, Tourette syndrome, Huntington's disease, Parkinson's disease and autism spectrum disorder (ASD).

36. A method for producing a human midbrain spheroid or organoid, the method comprising:

- (a) inducing a human pluripotent stem cell in suspension culture to a neural fate to provide a neural spheroid;
- (b) differentiating the neural spheroid into a midbrain spheroid; and
- (c) maintaining the midbrain spheroid in neural medium such that the midbrain spheroid comprises human midbrain neurons.

37. The method of claim 36, wherein the human midbrain neurons comprise dopaminergic neurons, including substantia nigra.

38. The method of claim 36 or claim 37, wherein the human pluripotent stem cell is an induced human pluripotent stem cell.

39. The method of any of claims 35-38, wherein inducing the human pluripotent stem cell in suspension culture to the neural fate in step (a) comprises culturing in a medium comprising an inhibitor of bone morphogenetic protein (BMP), an inhibitor of transforming growth factor β (TGF β), FGF8, and a sonic hedgehog pathway agonist.

40. The method of claim 39, wherein the inhibitor of BMP is dorsomorphin and the inhibitor of TGF β is SB-431542.

41. The method of claim 39, wherein the sonic hedgehog pathway agonist is smoothened agonist (SAG).

42. The method of any of claims 36-41 wherein step (a) comprises culturing in a medium comprising the inhibitor of BMP and inhibitor of TGF β is for a period of from 4 to 10 days, and adding the FGF8 and sonic hedgehog pathway agonist to the medium after about 3 days of culture in the medium comprising the inhibitor of BMP and inhibitor of TGF β .

43. The method of claim 42, wherein the FGF8 and sonic hedgehog pathway agonist are maintained for a period of about 1 day.

44. The method of any of claims 36-43, wherein differentiating the neural spheroid into a midbrain spheroid in step (b) comprises:

transferring the neural spheroid to a suspension culture in neural medium supplemented with FGF8, a sonic hedgehog pathway agonist, an inhibitor of BMP and an inhibitor of GSK-3; and

culturing the neural spheroid in suspension culture in neural medium supplemented with brain-derived neurotrophic factor (BDNF), NT3, L-Ascorbic Acid, cAMP, DHA, and DAPT

45. The method of claim 44, wherein the sonic hedgehog pathway agonist is smoothened agonist (SAG), the inhibitor of BMP is LND 193189 and the inhibitor of GSK-3 is CHIR99021.

46. A midbrain spheroid comprising human midbrain neurons, wherein the midbrain spheroid is capable of being maintained in suspension culture without adhering to a surface for at least 1 month.

47. A midbrain spheroid obtained by the method of any of claims 36-45.

48. A method for producing a midbrain-striatal assembloid *in vitro*, the method comprising:

(i) (a) inducing a first human pluripotent stem cell in suspension culture to a neural fate to provide a first neural spheroid;

(b) differentiating the first neural spheroid into a striatal spheroid (hStrS),

(ii) (a) inducing a second human pluripotent stem cell in a suspension culture to a neural fate to provide a second neural spheroid;
(b) differentiating the second neural spheroid into a midbrain spheroid (hMbS), and
(iii) culturing the hStrS and hMbS under conditions permissive for cell fusion in neural medium, such that the midbrain-striatal assembloid comprises human midbrain neurons projecting from the hMbS into the hStrS.

49. The method of claim 48, wherein the human midbrain neurons comprise dopaminergic neurons.

50. The method of claim 48 or 49, wherein inducing the first pluripotent stem cell in suspension culture to the neural fate in step (i)(a) comprises culturing in a medium comprising an inhibitor of bone morphogenetic protein (BMP) and an inhibitor of transforming growth factor β (TGF β).

51. The method of any of claims 48-50, wherein culturing in the medium comprising the inhibitor of BMP and inhibitor of TGF β is for a period of from 2 to 10 days.

52. The method of any of claims 48-51, wherein inducing the second pluripotent stem cell in suspension culture to the neural fate in step (ii)(a) comprises culturing in a medium comprising an inhibitor of bone morphogenetic protein (BMP), an inhibitor of transforming growth factor β (TGF β), FGF8, and a sonic hedgehog pathway agonist.

53. The method of any of claims 48-52, wherein step (ii)(a) comprises culturing in a medium comprising the inhibitor of BMP and inhibitor of TGF β is for a period of from 4 to 10 days, and adding the FGF8 and sonic hedgehog pathway agonist to the medium after about 3 days of culture in the medium comprising the inhibitor of BMP and inhibitor of TGF β ,
optionally wherein the FGF8 and sonic hedgehog pathway agonist are maintained for a period of about 1 day.

54. The method of any claim 52 or claim 53, wherein the sonic hedgehog pathway agonist is smoothened agonist (SAG).

55. The method of any one of claims 52 to 54, wherein the inhibitor of BMP is dorsomorphin and the inhibitor of TGF β is SB-431542.

56. The method of any one of claims 52 to 55, wherein culturing in the medium comprising the inhibitor of BMP and inhibitor of TGF β is for a period of from 2 to 10 days.

57. The method of any of claims 48-56, wherein differentiating the first neural spheroid into the hStrS in step (i)(b) comprises culturing the first neural spheroid in suspension culture in neural medium comprising an inhibitor of the Wnt pathway, activin A, and a retinoid X receptor (RXR) agonist.

58. The method of claim 57, wherein the first neural spheroid is cultured in neural medium comprising the inhibitor of the Wnt pathway and the activin A for a period of 4 to 8 weeks, and wherein the neural medium is supplemented with the RXR agonist after a period of 2 to 10 days.

59. The method of any of claims 48-58, wherein differentiating the second neural spheroid into the hMbS in step (ii)(b) comprises

transferring the neural spheroid to a suspension culture in neural medium supplemented with FGF8, a sonic hedgehog pathway agonist, an inhibitor of BMP and an inhibitor of GSK-3; and

culturing the neural spheroid in suspension culture in neural medium supplemented with brain-derived neurotrophic factor (BDNF), NT3, L-Ascorbic Acid, cAMP, DHA, and DAPT

60. The method of claim 59, wherein the sonic hedgehog pathway agonist is smoothened agonist (SAG), the inhibitor of BMP is LND 193189 and the inhibitor of GSK-3 is CHIR99021.

61. The method of any of claims 48-60, wherein the hStrS and hMbS are cultured under conditions permissive for cell fusion for at least 10 days.

62. The method of claim 61, wherein culturing the hStrS and hMbS under conditions permissive for cell fusion comprises culturing the hStrS and hMbS in close proximity to each other.

63. The method of any of claims 48-62, wherein the suspension culture of step (i)(a) and/or step (ii)(b) is feeder layer free.

64. The method of any of claims 48-63, wherein cells of the midbrain-striatal assembloid comprise at least one allele associated with a neurologic or psychiatric disorder, optionally wherein the neurologic or psychiatric disorder is selected from the group consisting of: schizophrenia, obsessive-compulsive disorder, Tourette syndrome, Huntington's disease, Parkinson's disease and autism spectrum disorder (ASD).

65. A midbrain-striatal assembloid comprising a striatal spheroid (hStrS) fused to a midbrain spheroid (hMbS), wherein the midbrain-striatal assembloid comprises human midbrain neurons projecting from the hMbS into the hStrS, and wherein the midbrain-striatal assembloid is capable of being maintained in suspension culture without adhering to a surface for at least 4 weeks.

66. A midbrain-striatal assembloid produced by the method of any of claims 48-64.

67. A method of determining the effect of a candidate agent on striato-midbrain neural circuits, the method comprising: contacting the candidate agent with the midbrain-striatal assembloid of claim 65 or claim 66; and determining the effect of the candidate agent on the number and/or function of striato-midbrain neural circuits in the midbrain-striatal assembloid,

optionally wherein cells of the midbrain-striatal assembloid comprise at least one allele associated with a neurologic or psychiatric disorder, further optionally wherein the neurologic or psychiatric disorder is selected from the group consisting of: schizophrenia, obsessive-compulsive disorder, Tourette syndrome, Huntington's disease, Parkinson's disease and autism spectrum disorder (ASD).