

THAT WHICH IS CLAIMED IS:

1. A method for producing a human raphe nuclei-like spheroid or organoid (hRNS) *in vitro*, the method comprising:
 - (a) inducing a human pluripotent stem cell in 3D suspension culture to a neural fate to generate a neural spheroid;
 - (b) differentiating the neural spheroid into a hRNS; and
 - (c) maintaining the hRNS in neural medium, such that the hRNS comprises serotonergic neurons.
2. The method of claim 1, wherein the human pluripotent stem cell is an induced human pluripotent stem cell.
3. The method of claim 1, 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), and wherein the method comprises supplementing the medium with an inhibitor of GSK-3.
4. The method of claim 3, wherein the inhibitor of BMP is dorsomorphin and the inhibitor of TGF β is SB-431542.
5. The method of claim 3 or claim 4, wherein the inhibitor of GSK-3 is CHIR99021.
6. The method of claim 3, 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.
7. The method of claim 6, wherein the medium is supplemented with the inhibitor of GSK-3 after a period of 1 to 2 days such that the medium comprises the inhibitor of BMP, inhibitor of TGF β and inhibitor of GSK-3, optionally wherein culturing in the medium comprising the inhibitor of BMP, inhibitor of TGF β and inhibitor of GSK-3 is for a period of from 4 to 8 days.
8. The method of claim 7, wherein culturing in the medium comprising the inhibitor of BMP, inhibitor of TGF β and inhibitor of GSK-3 comprises:

(1) culturing in a medium comprising the inhibitor of BMP, the inhibitor of TGF β , and inhibitor of GSK-3 for a period of 2 to 5 days, wherein the inhibitor of TGF β is present at a concentration of between 5 μ M to 20 μ M; and subsequently

(2) culturing in a medium comprising the inhibitor of BMP, the inhibitor of TGF β , and inhibitor of GSK-3 for a period of 2 to 5 days, wherein the inhibitor of TGF β is present at a concentration of between 1 μ M to 5 μ M.

9. The method of claim 1, wherein the suspension culture of step (a) is feeder layer free.

10. The method of claim 1, wherein differentiating the neural spheroid into the hRNS of step (b) comprises culturing the neural spheroid in suspension culture in neural medium comprising an inhibitor of GSK-3 and a sonic hedgehog pathway agonist, and wherein the method comprises supplementing the neural medium with FGF4.

11. The method of claim 10, wherein the inhibitor of GSK-3 is CHIR99021.

12. The method of claim 10 or claim 11, wherein the sonic hedgehog (SHH) pathway agonist is smoothened agonist (SAG).

13. The method of claim 10, wherein the neural spheroid is cultured in neural medium comprising the inhibitor of GSK-3 and a SHH pathway agonist for a period of 1 to 3 weeks, and wherein the neural medium is supplemented with FGF4 after a period of 2 to 10 days.

14. The method of claim 1, 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 1 to 3 weeks.

15. The method of claim 1, wherein differentiating the neural spheroid into the hRNS of step (b) comprises:

(1) culturing the neural spheroid in suspension culture in neural medium comprising an inhibitor of GSK-3 and a sonic hedgehog pathway agonist for a period of 2 to 10 days;

(2) supplementing the neural medium with FGF4; and

(3) culturing the neural spheroid in suspension culture in neural medium comprising an inhibitor of GSK-3, sonic hedgehog pathway agonist and FGF4 for a period of 1 to 3 weeks.

16. The method of claim 15, further comprising following step (3):

(4) culturing the neural spheroid in suspension culture for a period of 2 to 10 days in neural medium comprising FGF4 and 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-Dibutyryladenosine 3', 5' -cyclic monophosphate sodium salt (cAMP), cis-4, 7, 10, 13,16, 19- Docosahexaenoic acid (DHA), and DAPT; and

(5) culturing the neural spheroid in suspension culture for at least 1 week in neural medium comprising the at least one compound in the absence of FGF4.

17. The method of claim 1, wherein maintaining the hRNS in step (c) is carried out in neural medium in the absence of growth factors.

18. The method of claim 1, wherein maintaining the hRNS in step (c) is for at least 1 week.

19. The method of claim 1, wherein cells of the hRNS comprise at least one allele associated with a neurologic or psychiatric disorder.

20. The method of claim 17, wherein the neurologic or psychiatric disorder is selected from the group consisting of: schizophrenia, affective disorder and autism spectrum disorder (ASD), optionally wherein the affective disorder is major depressive disorder, bipolar disorder or an anxiety disorder.

21. A human raphe nuclei spheroid (hRNS) comprising human serotonergic neurons, wherein the hRNS is capable of being maintained in suspension culture without adhering to a surface for at least 1 month.

22. A human raphe nuclei spheroid (hRNS) obtained by the method of claim 1.

23. A method for producing a cortico-raphe nuclei assembloid (hCS-hRNS) *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 human raphe nuclei spheroid (hRNS),

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

(b) differentiating the second neural spheroid into a cortical spheroid (hCS), and

(iii) culturing the hRNS and hCS under conditions permissive for cell fusion in neural medium, such that the cortico-raphe assembloid comprises human serotonergic neurons with projections between the hRNS and hCS, and neurons from the hCS projecting into hRNS.

24. The method of claim 23, wherein human neurons form bidirectional projections between the hRNS and hCS.

25. The method of claim 23, wherein the first and/or second human pluripotent stem cell is an induced human pluripotent stem cell.

26. The method of claim 23, wherein inducing the first human 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), and wherein the method comprises supplementing the medium with an inhibitor of GSK-3.

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

28. The method of claim 23 - 26, wherein the inhibitor of GSK-3 is CHIR99021.

29. The method of claim 23, 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.

30. The method of claim 29, wherein the medium is supplemented with the inhibitor of GSK-3 after a period of 1 to 2 days such that the medium comprises the inhibitor of BMP, inhibitor of TGF β and inhibitor of GSK-3, optionally wherein culturing in the medium comprising the inhibitor of BMP, inhibitor of TGF β and inhibitor of GSK-3 is for a period of from 4 to 8 days.

31. The method of claim 30, wherein culturing in the medium comprising the inhibitor of BMP, inhibitor of TGF β and inhibitor of GSK-3 comprises:

(1) culturing in a medium comprising the inhibitor of BMP, the inhibitor of TGF β , and inhibitor of GSK-3 for a period of 2 to 5 days, wherein the inhibitor of TGF β is present at a concentration of between 5 μ M to 20 μ M; and subsequently

(2) culturing in a medium comprising the inhibitor of BMP, the inhibitor of TGF β , and inhibitor of GSK-3 for a period of 2 to 5 days, wherein the inhibitor of TGF β is present at a concentration of between 1 μ M to 5 μ M.

32. The method of claim 23, wherein differentiating the first neural spheroid into the hRNS of step (i)(b) comprises culturing the neural spheroid in suspension culture in neural medium comprising an inhibitor of GSK-3 and a SHH pathway agonist, and wherein the method comprises supplementing the neural medium with FGF4.

33. The method of claim 32, wherein the inhibitor of GSK-3 is CHIR99021.

34. The method of claim 32-33, wherein the SHH pathway agonist is smoothened agonist (SAG).

35. The method of claim 32, wherein the neural spheroid is cultured in neural medium comprising the inhibitor of GSK-3 and a sonic hedgehog pathway agonist for a period of 1 to 3 weeks, and wherein the neural medium is supplemented with FGF4 after a period of 2 to 10 days.

36. The method of claim 23, wherein step (i)(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), NT-3, L-Ascorbic Acid 2-phosphate Trisodium Salt (AA), N6, 2'-O-Dibutyryladenosine 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 1 to 3 weeks.

37. The method of claim 23, wherein differentiating the first neural spheroid into the hRNS of step (i)(b) comprises:

- (1) culturing the neural spheroid in suspension culture in neural medium comprising an inhibitor of GSK-3 and a sonic hedgehog pathway agonist for a period of 2 to 10 days;
- (2) supplementing the neural medium with FGF4; and
- (3) culturing the neural spheroid in suspension culture in neural medium comprising an inhibitor of GSK-3, sonic hedgehog pathway agonist and FGF4 for a period of 1 to 3 weeks.

38. The method of claim 37, further comprising following step (3):

- (4) culturing the neural spheroid in suspension culture for a period of 2 to 10 days in neural medium comprising FGF4 and 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-Dibutyryladenosine 3', 5' -cyclic monophosphate sodium salt (cAMP), cis-4, 7, 10, 13, 16, 19- Docosahexaenoic acid (DHA), and DAPT; and
- (5) culturing the neural spheroid in suspension culture for at least 1 week in neural medium comprising the at least one compound in the absence of FGF4.

39. The method of claim 23, wherein step (iii) comprises culturing the hRNS and hCS are cultured under conditions permissive for cell fusion for at least 3 days.

40. The method of claim 23, wherein step (iii) comprises culturing the hRNS and hCS in direct physical contact to each other.

41. The method of claim 23, wherein the suspension culture of step (i)(a) and/or step (ii)(a) is feeder layer free culture condition.

42. The method of claim 23, wherein cells of the cortico-raphé nuclei assembloid comprise at least one allele or genetic event associated with a neurologic or psychiatric disorder.

43. The method of claim 23, wherein either the first or second human pluripotent stem cell comprises at least one allele or genetic event associated with a neurologic or psychiatric disorder.

44. The method of claim 42-43, wherein the neurologic or psychiatric disorder is selected from the group consisting of: schizophrenia, affective disorder and autism spectrum disorder (ASD), optionally wherein the affective disorder is major depressive disorder, bipolar disorder or an anxiety disorder.

45. A cortico-raphé nuclei assembloid (hCS-hRNS) comprising a raphe nuclei spheroid (hRNS) fused to a cortical spheroid (hCS), wherein the hCS-hRNS comprises human serotonergic neurons projecting between the hRNS and hCS and forming a human neuromodulatory circuit, and wherein the hCS-hRNS is capable of being maintained in suspension culture without adhering to a surface for at least 4 weeks.

46. A cortico-raphé nuclei assembloid produced by the method of any of claims 23-44.

47. A method of determining the effect of a candidate agent on serotonergic modulation on cortical neural circuits, the method comprising: contacting the candidate agent with the cortico-raphé nuclei assembloid (hCS-hRNS) of claim 45-46; and determining the effect of the candidate agent on the ability of serotonergic neurons to modulate function of cortical neural circuits,

optionally wherein cells of the hCS-hRNS comprise at least one allele or genetic event associated with a neurologic or psychiatric disorder, further optionally wherein the neurologic or psychiatric disorder is selected from the group consisting of: schizophrenia, affective disorder and autism spectrum disorder (ASD).

48. The method of claim 47, wherein the candidate agent is a selective serotonin reuptake inhibitor (SSRI).