HUMAN CELLULAR MODEL FOR INVESTIGATING CORTICO-STRIATAL-MIDBRAIN NEURAL PATHWAYS

CROSS REFERENCE TO RELATED APPLICATIONS

[001] This application claims priority to U.S. Provisional Application No. 62/928,905, filed October 31, 2019, which is incorporated herein in its entirety for all purpose.

BACKGROUND OF THE INVENTION

[002] Neural projections from pyramidal neurons in the cerebral cortex towards the striatum are essential components of the neural circuits controlling motivated behavior. This connectivity between the cerebral cortex and the striatum is unidirectional with pyramidal cortical neurons projecting into striatum, and then medium spiny neurons in the striatum subsequently connecting to midbrain dopaminergic neurons as part of the direct and indirect basal ganglia pathways. Dysfunction in this cortico-striatal pathway is thought to contribute to severe neuropsychiatric disorders such as schizophrenia, obsessive-compulsive disorder, Tourette syndrome, Huntington's disease and autism spectrum disorder (ASD) (Shepherd and Gordon, 2013).

[003] However, the specific molecular and cellular mechanisms leading to disease in these patients are still not fully understood mostly due to the inaccessibility to direct investigation of these pathways in the intact, functional human brain and challenges with translating findings from animal models.

[004] Therefore, there is urgent need to develop human cellular models that realistically capture neural circuits formation, function and dysfunction (Pasca, 2018).

SUMMARY ~~OF THE INVENTION~~

[005] Compositions and methods are provided for *in vitro* generation of human striatal organoids or spheroids (hStrS) and human midbrain organoids or spheroids (hMbS), which may be generated entirely from human pluripotent stem (hPS) cells. Such spheroids, which may also be referred to as brain region-specific or regionalized organoids, represent the human striatum (basal ganglia) and human midbrain (including substantia nigra) and contain specific sets of cells (e.g. neurons, glial cells) that are associated with the cortex, striatum (basal ganglia) and midbrain of a human.

[006] The hStrS can be functionally integrated with human cerebral cortical spheroids (hCS), which include human cortical neurons such as glutamatergic pyramidal neurons, to provide a cortico-striatal assembloids (hCS-hStrS). Functionally integrated cells interact in a physiologically relevant manner, *e.g.* forming synapses, releasing neurotransmitters. Human cortical neurons in the hCS-hStrS project from the hCS to the hStrS forming functional cortico-striatal neural circuits. Using a combination of viral tracing and live imaging, evidence is

provided herein for the formation of an *in vitro* generated human cortico-striatal neural circuit, which provides useful modeling of cortico-striatal pathway development and dysfunction.

[007] The hStrS can also be functionally integrated with hMbS to provide a midbrain-striatal assembloid (hMbS-hStrS). Human midbrain neurons, e.g. dopaminergic neurons, project from the midbrain into the hStrS forming striato-midbrain neural circuits. Dopaminergic inputs from the midbrain to the striatum (basal ganglia) are thought to play a crucial role in the development and maturation of the striatum and therefore these assembloids provide unique opportunities to study development and dysfunction of the striatum. Striatal neurons in hStrS also project into the hMbS to provide GABAergic modulation of midbrain dopaminergic neurons. Furthermore, hMbS can be combined with the hCS and hStrS to form assembloids containing cortico-striatal and striato-midbrain neural circuits, therefore allowing for the concurrent study of glutamatergic and dopaminergic input into the striatum (basal ganglia).

[008] The assembloids, i.e. hCS-hStrS, hMbS-hStrS and assembloids comprising hCS, hStrS and hMbS, provide unique opportunities for analysis of the development and function of neural circuits between the human cerebral cortex, striatum and midbrain. Furthermore, these assembloids provide a model to study how specific neurologic or psychiatric disorders impair these neural circuits. Of particular relevance are those neurologic or psychiatric disorders that are associated with cortico-striatal dysfunction, such as schizophrenia, obsessive-compulsive disorder, Tourette syndrome, Parkinson's disease, Huntington's disease and ASD.

[008a] In one aspect, the present disclosure provides a method for producing a human striatal 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, optionally wherein the pluripotent stem cell is an induced pluripotent stem cell; (b) differentiating the neural spheroid into a striatal spheroid; and (c) maintaining the striatal spheroid in neural medium, such that the striatal spheroid comprises human striatal neurons.

[008b] In another aspect, the present disclosure provides a striatal spheroid obtained by the method disclosed herein.

[008c] In yet another aspect, the present disclosure provides a method for producing a cortico-striatal assembloid *in vitro*, the method comprising: (i)(a) inducing a human pluripotent stem cell in a suspension culture to a neural fate to provide a neural spheroid; (b) differentiating the neural spheroid into a cortical spheroid (hCS), and (ii) culturing the hStrS disclosed herein 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.

[008d] In one aspect, the present disclosure provides a cortico-striatal assembloid produced by the method disclosed herein.

[008e] In another aspect, the present disclosure provides 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 disclosed herein; 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).

[008f] In yet another aspect, the present disclosure provides a method for producing a midbrain-striatal assembloid *in vitro*, the method comprising:

(i) (a) inducing a human pluripotent stem cell in a suspension culture to a neural fate to provide a neural spheroid; (b) differentiating the neural spheroid into a midbrain spheroid (hMbS), and (ii) culturing the hStrS disclosed herein 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.

[008g] In one aspect, the present disclosure provides a midbrain-striatal assembloid produced by the method disclosed herein.

[008h] In another aspect, the present disclosure provides 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 disclosed herein; 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).

[008i] In yet another aspect, the present disclosure provides a midbrain spheroid obtained by the method disclosed herein.

[009] In some ~~embodiment~~examples, assembloids are provided in which one or more of the cells have been genetically modified to provide for additional functionality in screening. For example, one or more of cortical neurons, striatal neurons and midbrain neurons can be genetically modified to express a fluorescent calcium indicator, which indicators are known and used in the art. One or more of cortical neurons, striatal neurons (medium spiny neurons) and midbrain neurons (dopaminergic neurons) can be genetically modified to express a light activated opsin. In some ~~embodiment~~examples, an assembloid comprises neurons expressing an opsin, and other neurons expressing a fluorescent calcium indicator, where a functional relationship between the neurons is demonstrated by using light to activate a neuron, and observing a calcium indicator response from another neuron.

[0010] Also provided are spheroids, i.e. hStrS and hMbS, and methods of making said spheroids, as further defined herein. Also provided are assembloids, i.e. hCS-hStrS, hMbS-hStrS and assembloids comprising hCS, hStrS and hMbS, and methods of making said assembloids, as further defined herein.

[0011] Also provided are methods for determining the activity of a candidate agent neural circuits in the assembloids, the method comprising contacting the candidate agent with the assembloid. The cells present in the assembloid optionally comprise at least one allele encoding a mutation associated with, or potentially associated with, a neurologic or psychiatric disorder, and determining the effect of the agent on morphological, genetic or functional

parameters, including without limitation neuron number, neuron function, gene expression profiling, cell death, single cell gene expression (RNA-seq), calcium imaging with pharmacological screens, patch-clamp recordings, modulation of synaptogenesis, and the like.

[0012] These and other objects, advantages, and features of the ~~invention~~disclosure will become apparent to those persons skilled in the art upon reading the details of the subject methods and compositions as more fully described below.

BRIEF DESCRIPTION OF THE DRAWINGS

[0013] The invention is best understood from the following detailed description when read in conjunction with the accompanying drawings. It is emphasized that, according to common practice, the various features of the drawings are not to-scale. On the contrary, the dimensions of the various features are arbitrarily expanded or reduced for clarity. Included in the drawings are the following figures.

[0014] **FIG. 1A–1P**. **Generation of human striatal spheroids (hStrS) from human pluripotent stem cells** (**FIG. 1A**) Scheme illustrating the main stages for the generation of hStrS (also named or basal ganglia organoids) from human induced pluripotent stem (hiPS) cells and subsequent analysis by qPCR, sectioning and immunohistochemistry (IHC), single cell RNA-seq, live cell imaging and electrophysiological recordings (**FIG. 1B**) Levels of gene expression of forebrain marker *FOXG1* and lateral ganglionic eminence (LGE) markers *GSX2, CTIP2* and *MEIS2* in hStrS and human cortical spheroids (hCS) at day 23. Levels of gene expression were normalized to *GADPH*. $n = 9$ spheroids produced from 4 hiPS cell lines. (**FIG. 1C**) Immunostaining of hCS and hStrS at day 80 showing protein expression of DARPP32, GAD67, CTIP2. (**FIG. 1D, E**) Immunostaining of hStrS at day 85 showing protein expression of DARPP32, CTIP2 and NeuN and quantification. $n = 8$ spheroids produced from 3 hiPS cell lines. Arrowheads indicate cells expressing DARPP32 and NeuN, indicative of medium spiny neurons. (**FIG. 1F**) Single cell-RNA-sequencing analysis from approximately 25,772 cells dissociated from day 80-83 hStrS reveals distinct populations of GABAergic neurons, neural progenitor cells, astrocytes, oligodendrocytes and glutamatergic neurons. (**FIG. 1G**) Correlation to the BrainSpan dataset to the human developing brain (PCW 20–25). (**FIG. 1H**) Single cell-RNA-sequencing analysis reveals strong expression of genes enriched in forebrain (*FOXG1*) and LGE (*DLX1*, *DLX2*, *DLX5*, *DLX6*, *GSX2, ASCL1*, *FOXP1* and *FOXP2*) but not of genes in medial ganglionic eminence (MGE; *NKX2-1*) or in cerebral cortex (*EMX1*). (**FIG. 1I**) Representative image of a control hStrS cell transfected with AAV-mDlx::eGFP, showing *mDlx*::eGFP$^+$ cells develop dendritic spine-like structures at 65 days (in this case after dissociation and plating in 2D). (**FIG. 1J, K, L**) Representative image of hStrS transfected with AAV-mDlx::eGFP showing *mDlx*::eGFP$^+$ cells with dendritic spine-like structures in 3D

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, optionally wherein the pluripotent stem cell is an induced pluripotent stem cell;

(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, wherein the mature morphology has neurons with spines and wherein the electrophysiological properties are inward rectifications, and

optionally 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.

~~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~~3. The method of ~~any of claims 1-6~~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 (TGFβ), optionally wherein the medium further comprises an inhibitor of the Wnt pathway, optionally wherein the inhibitor of BMP is selected from the group of dorsomorphin, LDN-193189, and LY364947, and wherein the inhibitor of TGFβ is SB-431542,

optionally wherein culturing in the medium is for a period of from 2 to 10 days, and optionally wherein the suspension culture is feeder layer free; and

optionally 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, wherein the neural medium is supplemented with the RXR agonist after a period of 2 to 10 days, optionally wherein the neural medium is supplemented after a period of 3 to 4 weeks with at least one of the compound 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.

~~8. The method of claim 7, wherein the inhibitor of BMP is dorsomorphin and the inhibitor of TGFβ 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 TGFβ 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-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 3 to 4 weeks.~~

~~13~~4. The method of ~~any of claims 1-12~~claim 1, wherein maintaining the striatal spheroid in step (c) is carried out in neural medium in the absence of growth factors for at least 1 month, and optionally wherein cells of the striatal spheroid comprise at least one allele associated with a neurologic or psychiatric disorder selected from the group consisting of: schizophrenia, obsessive-compulsive disorder, Tourette syndrome, Huntington's disease, Parkinson's disease and autism spectrum disorder (ASD).

~~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~~5. A striatal spheroid obtained by the method of ~~any of claims 1-16~~claim 1.

~~19~~6. 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),~~

(i~~i~~)(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

(ii~~i~~) culturing the hStrS of claim 1 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~~7. The method of claim ~~19~~6, wherein the human cortical neurons comprise neurons expressing CTIP2 and/or neurons expressing SATB2, which can project and connect specifically with medium spiny neurons, and wherein the hStrS comprises human striatal neurons, optionally wherein the human striatal neurons comprise medium spiny neurons, and optionally wherein the assembloid comprises a functional neural circuit between the human cortical neurons and human striatal 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~~8. The method of ~~any of claims 19-22~~claim 6, wherein inducing the ~~first and second~~ human pluripotent stem cell in suspension culture to the neural fate in step~~s~~ (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 (TGFβ) for 2 to 10 days,
optionally wherein the medium further comprises an inhibitor the Wnt pathway, and optionally wherein the inhibitor of BMP is selected from the group of dorsomorphin, LDN-193189, and LY364947, and wherein the inhibitor of TGFβ is SB-431542; and

optionally wherein differentiating the neural spheroid into the hCS in step (i)(b) comprises:

culturing the neural spheroid in suspension culture in medium comprising fibroblast growth factor 2 (FGF2) and epidermal growth factor (EGF) to provide cortical progenitor spheroids for a period of from 1 to 4 weeks; 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-Dibutyryladenosine 3', 5' -cyclic monophosphate sodium salt (cAMP), cis-4, 7, 10, 13, 16, 19- Docosahexaenoic acid (DHA) for a period of from 1 to 4 weeks; and

optionally wherein step (ii) comprises culturing the hStrS and hCS under conditions permissive for cell fusion for at least 10 days, optionally wherein the culturing the hStrS and hCS is done in close proximity to each other, and optionally wherein the suspension culture is feeder layer free.

~~24. The method of claim 23, wherein the inhibitor of BMP is dorsomorphin and the inhibitor of TGFβ 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 TGFβ 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-Dibutyryladenosine 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~~9. A cortico-striatal assembloid produced by the method of ~~any of claims 19-32~~claim 6.

~~35~~10. 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~~9; 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 (TGFβ), FGF8, and a sonic hedgehog pathway agonist.

40. The method of claim 39, wherein the inhibitor of BMP is dorsomorphin and the inhibitor of TGFβ 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 TGFβ 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 TGFβ.

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~~11. 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),~~

(i~~i~~) (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

(ii~~i~~) culturing the hStrS of claim 1 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 (TGFβ).~~

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

~~52~~12. The method of ~~any of claims 48-51~~claim 11, wherein inducing the ~~second~~ pluripotent stem cell in suspension culture to the neural fate in step (i~~i~~)(a) comprises culturing in a medium comprising an inhibitor of bone morphogenetic protein (BMP), an inhibitor of transforming growth factor β (TGFβ), FGF8, and a sonic hedgehog pathway agonist, optionally wherein the inhibitor of BMP is selected from the group of dorsomorphin, LDN-193189, and LY364947, wherein the inhibitor of TGFβ is SB-431542 and wherein the sonic hedgehog pathway agonist is smoothened agonist (SAG), optionally wherein culturing in a medium comprising the inhibitor of BMP and inhibitor of TGFβ 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 TGFβ, optionally wherein the FGF8 and sonic hedgehog pathway agonist are maintained for a period of about 1 day; and

optionally wherein differentiating the neural spheroid into the hMbS in step (i)(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, optionally 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; and

optionally wherein the hStrS and hMbS are cultured under conditions permissive for cell fusion for at least 10 days, optionally wherein the culturing the hStrS and hMbS are cultured in close proximity to each other, and optionally wherein the suspension culture is feeder layer free.

~~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 TGFβ 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 TGFβ,~~

~~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 TGFβ 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 TGFβ 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~~13. A midbrain-striatal assembloid produced by the method of ~~any of claims 48-64~~claim 11.

~~67~~14. 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~~13; 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).

15. A midbrain spheroid obtained by the method of claim 11.