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 in a medium to a neural fate to provide a neural spheroid, wherein the medium comprises two SMAD pathway inhibitors, optionally further comprises an inhibitor of the Wnt pathway, and optionally wherein the pluripotent stem cell is an induced pluripotent stem cell; (b) differentiating the neural spheroid into a striatal spheroid in suspension culture in a neural medium comprising an inhibitor of the Wnt pathway, an activin A, and a retinoid X receptor (RXR) agonist; 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 in a medium to provide a neural spheroid, wherein the medium comprises an inhibitor of bone morphogenetic protein (BMP) and an inhibitor of transforming growth factor β (TGFβ); optionally wherein the medium further comprises an inhibitor the Wnt pathway; (b) differentiating the neural spheroid into a cortical spheroid (hCS) in suspension culture in medium comprising fibroblast growth factor 2 (FGF2) and epidermal growth factor (EGF), 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 wherein the medium comprises at least two SMAD inhibitors, a fibroblast growth factor (FGF), and a sonic hedgehog pathway agonist; (b) differentiating the neural spheroid into a midbrain spheroid (hMbS) in a neural medium supplemented with FGF8, a sonic hedgehog pathway agonist, an inhibitor of BMP and an inhibitor of GSK-3, 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 example, the present disclosure provides a midbrain spheroid obtained by the method disclosed herein.

[009] In some 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 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

That which is claimed is:

1. 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 in a medium to a neural fate to provide a neural spheroid, wherein the medium comprises two SMAD pathway inhibitors, optionally further comprises an inhibitor of the Wnt pathway, and optionally wherein the pluripotent stem cell is an induced pluripotent stem cell;

(b) differentiating the neural spheroid into a striatal spheroid in suspension culture in a neural medium comprising an inhibitor of the Wnt pathway, an activin A, and a retinoid X receptor (RXR) agonist; 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 1, wherein the two SMAD pathway inhibitors of step a) comprise an inhibitor of bone morphogenetic protein (BMP) and an inhibitor of transforming growth factor β (TGFβ), wherein the inhibitor of BMP is selected from the group of dorsomorphin, LDN-193189, and LY364947, and wherein the inhibitor of TGFb 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 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.

4. The method of 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).

5. A striatal spheroid obtained by the method of claim 1.

6. 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 in a medium to provide a neural spheroid, wherein the medium comprises an inhibitor of bone morphogenetic protein (BMP) and an inhibitor of transforming growth factor β (TGFβ); optionally wherein the medium further comprises an inhibitor the Wnt pathway;

(b) differentiating the neural spheroid into a cortical spheroid (hCS) in suspension culture in medium comprising fibroblast growth factor 2 (FGF2) and epidermal growth factor (EGF), and

(ii) 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 and wherein the assembloid comprises a functional neural circuit between the human cortical neurons and human striatal neurons.

7. The method of claim 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.

8. The method of claim 6, wherein inducing the human pluripotent stem cell in suspension culture to the neural fate in step (i)(a) comprises culturing in the medium for 2 to 10 days,

wherein the inhibitor of BMP is selected from the group of dorsomorphin, LDN-193189, and LY364947, and wherein the inhibitor of TGFb is SB-431542; and

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

culturing the neural spheroid 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.

9. A cortico-striatal assembloid produced by the method of claim 6.

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 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).

11. 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 wherein the medium comprises at least two SMAD inhibitors, a fibroblast growth factor (FGF), and a sonic hedgehog pathway agonist;

(b) differentiating the neural spheroid into a midbrain spheroid (hMbS) in a neural medium supplemented with FGF8, a sonic hedgehog pathway agonist, an inhibitor of BMP and an inhibitor of GSK-3, and

(ii) 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.

12. The method of claim 11, wherein the at least two SMAD inhibitors are an inhibitor of bone morphogenetic protein (BMP), and 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 TGFb 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

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.

13. A midbrain-striatal assembloid produced by the method of claim 11.

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 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).