Table S1: 3D *in vitro* human model systems and their applications for investigating neurodevelopmental disorders

Type of organoid	Modelled brain area	Technical aids	Added factors	Protocol advantages	Modelled disorder/phenotype	Identified mechanism	Reference
Undirected	Forebrain (dorsal and ventral, including forebrain organizing centers), midbrain, hindbrain, midbrain-hindbrain boundary, choroid plexus, retina; mesoderm	Matrigel embedding, spinning bioreactor	-	Generation of a variety of brain regions allows exploring human CNS diversity; recapitulation of temporal order of generation of subsequent cell types including neurons, astrocytes and oligodendrocytes. Clearing and 3D reconstruction shows interconnected ventricles (Renner et al., 2017).	**Microcephaly: *CDK5RAP2***	Premature neuronal differentiation: altered spindle orientation of radial glial cells, abundant neuronal outgrowth, smaller organoid and neuroepithelial region size due to decrease in proliferating progenitors	(Lancaster and Knoblich, 2014; Lancaster et al., 2013; Renner et al., 2017)
Undirected	Forebrain, midbrain, hindbrain	Chemically defined hydrogel material, chemically defined culture medium, static culture	-	Generation of a variety of brain regions allows to explore human CNS diversity; chemically defined medium simplifies scalability			(Lindborg et al., 2016)
Undirected	Forebrain, midbrain, hindbrain	Matrigel embedding, spinning bioreactor	-	Culture of sliced organoids shows generation of mature cortical neurons with extended long-range axons with complex branching and projections; also dendritic spines of GABAergic nature are produced	**Microcephaly + Seckel syndrome: *CPAP***	Increase in aRG with vertical cleavage plane, prematurely switching to asymmetric, neurogenic division; increased neuronal differentiation; increased number and length of cilia in aRG show defective cilium disassembly. → Role of cilium regulation in the maintenance of the stem cell pool	(Gabriel et al., 2016) (based on Lancaster and Knoblich, 2014)
Undirected	Forebrain, midbrain, hindbrain	Matrigel embedding, spinning bioreactor	-	Combination with live imaging of organoid slices and sc-RNAseq.; protocol also functions for primate iPSCs: cytoarchitecture, cell type composition, and neurogenic gene expression programs of humans and chimpanzees are remarkably similar	**Differences in cortical development between human, chimpanzee and macaque**	Lengthening of prometa-metaphase in human apical progenitors compared to chimpanzee leads to more proliferative divisions; differentially expressed genes indicate longer neurogenic period in humans. → Cortical progenitor cell clonal output regulates primate cerebral cortex size.	(Mora-Bermúdez et al., 2016) (based on Lancaster and Knoblich, 2014)

Undirected	Forebrain, midbrain, hindbrain, retina (10 clusters of cell types); mesoderm		-	Generation of a variety of brain regions allows to explore human CNS diversity; generation of mature neurons and glia, synaptic junctions, actively firing and light-sensitive retinal cells			(Quadrato et al., 2017)
Undirected	Different regional identities	Matrigel embedding, injection of organoids into Ca-Alginate microfibers	-	Ca-Alginate microfibers increase neuroectoderm induction and reproducibility of organoids	**Fetal alcohol syndrome (microcephaly)**	Reduced cell proliferation, increased cell death; premature neural differentiation with hyper production of glutamatergic neurons; reduced neurite outgrowth; increased proportion of astrocytes; altered Wnt, MAPK and Hippo signalling pathways; ectopic neurons reminiscent of cobblestone lissencephaly	(Zhu et al., 2017)
Undirected	Forebrain, midbrain, hindbrain	Matrigel embedding, orbital shaker	-	Generation of a variety of brain regions allows to explore the role of a newly identified candidate gene	**PH: biallelic mutations in *MOB2* as candidate causative gene**	Increase in the amount of primary cilia per cell upon *MOB2* knockdown → Role of cilium maintenance in neuronal migration; MOB2 is part of the Hippo pathway and phosphorylates FLNA, thereby linking both known PH disease pathways	(O'Neill et al., 2018) (based on Lancaster and Knoblich, 2014)

Slightly directed	Mostly forebrain identity	PLGA fiber microfilaments, matrigel embedding, addition of liquid matrigel to culture medium, spinning bioreactor or orbital shaker	CHIR99021 (3d pulse)	Addition of microfilament fibers results in elongated embryoid bodies and enhanced neuroectoderm formation; generating mostly forebrain tissue while maintaining self-organizing capacity; improved reproducibility and tissue architecture; generation of a basal membrane due to matrigel in the medium: RG processes reach basal membrane and form radial units	**Fetal alcohol syndrome (PH and microcephaly)**	Smaller cortical regions and reduction or complete absence of CP; PH-like heterotopic cluster of neurons; migrating neurons lacking the leading process (required for normal radial migration to the CP), resulting in disruption of locomotion and formation of heterotopic clumps	(Lancaster et al., 2017) (based on Lancaster and Knoblich, 2014)
Slightly directed	Dorsal forebrain	Matrigel embedding, orbital shaker	Dorsomorphin	Generation of larger organoids with folded surface after *PTEN* deletion due to sustained cell-cycle re-entry, expansion of progenitors and delayed neuronal differentiation	**Zika virus-mediated microcephaly**	Zika infection of neural progenitors impairs cortical growth and folding. *PTEN* deletion enhances AKT signaling, causing expansion of VZ, iSVZ, delayed neuronal differentiation and oSVZ and folding	(Li et al., 2017) (based on Lancaster and Knoblich, 2014)
Directed	Dorsal forebrain	Manual rosette selection	bFGF, DKK1 , SB431542 and BMPRIA-Fc	Rosette-based spheroids enable the study of basic concepts of development and disease in a highly reproducible system		Most similar to 8-10 PCW fetal dorsal telencephalon; recapitulation of *in vivo* cytoarchitecture, formation of excitatory neurons of different layer identities, of GABAergic interneurons, of synapses	(Mariani et al., 2012) (based on Eiraku et al., 2008)
Directed	Neocortex	40% oxygen; 2% matrigel dissolved in medium	IWR1e and SB431542; ventralised by SAG	Rolling morphogenesis and curvature with rostro-caudal polarization; cellular complexity and organization resembles early second trimester; appearance of bRG			(Kadoshima et al., 2013)

Directed	Forebrain assembloids: dorsal telencephalon- and ventral telencephalon-like regions	Manual rosette selection	Noggin, DKK1, bFGF, EGF, BDNF, GDNF, ascorbic acid, cAMP	Telencephalic organoids recapitulate transcriptional programs present in mid-fetal human cortical development	**ASD and macrocephaly**	Increased progenitor cell proliferation through decrease in cell cycle length; enhanced synaptic maturation; overproduction of GABAergic inhibitory neurons due to overexpression of transcription factor FOXG1 → Role of FOXG1 in excitation/inhibition imbalance	(Mariani et al., 2015)
Directed	Dorsal forebrain		Dorsomorphin, SB431542; bFGF and EGF; BDNF and NT3	Recapitulate later, mid-fetal stages of post conceptual weeks 19-24 and contain both neurons and non-reactive astrocytes, as well as functional synapses and electrophysiological signatures of network activity; equal proportions of deep and superficial neurons are present in 2.5 months old organoids; greater neuronal maturation in 3D than in 2D			(Paşca et al., 2015)
Directed	Neocortex	1% Matrigel dissolved in medium	IWR1e and SB431542	Species-specific timing of generation of neurons with different layer identities is preserved in organoids; combination with 2D culture for easier identification of underlying mechanisms (clonal analysis)	**Differences in cortical development between human, chimpanzee and macaque**	2D: Faster maturation of macaque neurons than chimpanzee and human; protracted progenitor proliferation in primates: more symmetric divisions in human than macaque cortical progenitors and longer cell-cycle duration in human vs. chimpanzee progenitors	(Otani et al., 2016)

Directed	Forebrain/ midbrain/ hypothalamus	Matrigel embedding; miniaturized spinning bioreactors	Forebrain: Dorsomorphin, A83–01, WNT3A, CHIR99021, SB431542; Midbrain: LDN-193189, SB431542, SHH, FGF-8, purmorphamine, CHIR99021; Hypothalamus: LDN-193189, SHH, SB431542, WNT3A, 1-Thioglycerol, , purmorphamine	Spinning bioreactors improve oxygen and nutrient diffusion to reach larger viable organoids and enable drug testing; production of neurons of all six layer identities that are produced at conserved timing; production of bRG	**Zika virus-mediated microcephaly**	Infection of neural progenitors leads to increased cell death and reduced proliferation, resulting in decreased neuronal layer volume and organoid size resembling microcephaly; enlarged ventricle-like lumen	(Qian et al., 2016)
Directed	Forebrain assembloids: fusion of dorsal telencephalon and ventral telencephalon	Matrigel embedding of dorsally and ventrally pre-patterned organoids for their fusion; orbital shaker	Ventralisation: IWP2, SAG; Dorsal forebrain identity: cyclopamine A	Generation of a dorso-ventral axis enables recapitulation of ventral-to-dorsal GABAergic interneuron migration (timelapse-imaging) and interactions between different brain regions; generation of several interneuron subtypes		Used to study interneuron migration over long distances, decipher if specific molecules act in neuronal migration in cell-autonomous or non-cell-autonomous fashion; useful because many psychiatric diseases, such as schizophrenia, are thought to involve selective deficits in specific interneuron subpopulations; organoid fusion slice culture paradigm in which confocal time-lapse imaging can be used to analyze the short-term dynamics of neuronal cell migration	(Bagley et al., 2017) (modified from Lancaster and Knoblich, 2014)

Directed	Forebrain	Matrigel embedding; shaking culture; 40 % oxygen in the beginning, addition of 2 % liquid matrigel to the medium	Small molecules LDN-193189, A83-01 and IWR-1; plus GSK3β inhibitor CHIR99021 for rescue of Wnt phenotype		**Miller-Dieker-Syndrome (lissencephaly Type I)**	Patient-derived organoids are significantly reduced in size: switch from symmetric to asymmetric cell division of aRG causes reduced expansion and premature neurogenesis; alterations in microtubule network organization in aRG and disruption of cortical niche architecture, including altered expression of cell adhesion molecules; non-cell-autonomous disturbance of the N-cadherin/β-catenin signalling axis	(Iefremova et al., 2017) (based on Kadoshima et al., 2013 that was improved in Krefft et al., 2018)
Directed	Neocortex	40% oxygen; 1% matrigel dissolved in medium	Wnt inhibitor IWR1-ε and TGF-β inhibitor SB431542	Human genetic model may be more sensitive in recapitulating lissencephaly severity as organoids contain bRG and enable genome editing; New assays: culture of organoid slides on matrigel-coated surface enables live imaging of migrating neurons; culture of organoid slices on human fetal cortical tissue enables analysis of cell-autonomous vs non-cell-autonomous mechanisms	**Miller-Dieker-Syndrome (lissencephaly Type I)**	Defective saltatory neuronal migration (reduced speed because of reduced tension during nucleokinesis; more resting timepoints; more tortuous/ tumbling migration); smaller organoid size caused by severe apoptosis of neuroepithelial stem cells accompanied by increased horizontal cell divisions due to dysregulation of mitotic spindles, leading to premature neurogenesis and overproduction of deep-layer neurons; prolonged mitosis of bRG	(Bershteyn et al., 2017) (based on Kadoshima et al., 2013)
Directed	Neocortex	Manual rosette selection; culture of 1 organoid/well in 96-well-plate	SB431542, LDN193189, PD0325901, bFGF, FGF18	Increased reproducibility with one neocortical unit/organoid makes this model useful for pharmacological applications	**Prenatal cocaine-induced impaired brain growth**	Proliferation deficit of neuroepithelial progenitors; premature neuronal differentiation; reduced CP formation	(Lee et al., 2017)

Directed	Forebrain assembloids: fusion of dorsal telencephalon- and ventral telencephalon-like spheroids	Placing of differently patterned spheroids into a common conical tube for their fusion	<u>dorsal forebrain</u>: dorsomorphin, SB431542, EGF, bFGF, BDNF, NT3 <u>ventral forebrain:</u> dorsopmorhin, SB431542, IWP-2, EGF, bFGF, SAG, retinoic acid, allopreganolene, BDNF, NT3	Fusion of dorsal cortical spheroid containing mature glutamatergic projection neurons of all layer identities with ventral telencephalic spheroid containing several types of GABAergic interneurons enables analysis of saltatory interneuron migration from ventral to dorsal part; both compartments produce astrocytes and the subpallial spheroid also produces oligodendrocytes; membrane potentials more mature in assembloids than in single spheroids; synapse formation between GABAergic interneurons and cortical neurons	**Timothy syndrome: ASD and epilepsy**	Cell-autonomous defects in saltatory migration of Timothy-syndrome derived cortical interneurons that could be restored pharmacologically by modulating the mutated L-type calcium channel	(Birey et al., 2017)
Directed	Forebrain	Organoids on a chip: grown in a 150 µm thin micro compartment between a coverslip and a semi-permeable polycarbonate membrane, embedded in matrigel	Ascorbic acid, LIF, bFGF, TGFβ1, IWR, CHIR99021, PD0325901, BIRB796, SP600125, LDN193189	Micro compartment allows tissue expansion only in the x,y dimension and long-term live imaging through the coverslip; surface wrinkles reminiscent of gyri and sulci develop as result of opposing bending and stretching forces from apical surface contraction vs. nuclear swelling in S-phase at basal position of IKNM	**Lissencephaly Type I (*LIS1* +/-)**	Less convolutions in patient organoids based on IKNM differences: softer cells and reduced opposing forces	(Karzbrun et al., 2018)