Stem cell models of human synapse development and degeneration

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ABSTRACT **Many brain disorders exhibit altered synapse formation in development or synapse loss with age. To understand the complexities of human synapse development and degeneration, scientists now engineer neurons and brain organoids from human-induced pluripotent stem cells (hIPSC). These hIPSC-derived brain models develop both excitatory and inhibitory synapses and functional synaptic activity. In this review, we address the ability of hIPSC-derived brain models to recapitulate synapse development and insights gained into the molecular mechanisms underlying synaptic alterations in neuronal disorders. We also discuss the potential for more accurate human brain models to advance our understanding of synapse development, degeneration, and therapeutic responses.**

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INTRODUCTION

In less than a decade, human-induced pluripotent stem cells (hIPSCs) have revolutionized research into complex human disorders (Dolmetsch and Geschwind, 2011), from neurodevelopmental disorders such as schizophrenia (Brennand *et al.*, 2011; Windrem *et al.*, 2017), autism (Marchetto *et al.*, 2010, 2017; Krey *et al.*, 2013; Mariani *et al.*, 2015; Nestor *et al.*, 2016), and microcephaly (Lancaster *et al.*, 2013; Qian *et al.*, 2016) to neurodegenerative disorders such as Alzheimer's (Israel *et al.*, 2012; Lin *et al.*, 2018), Parkinson's (Miller *et al.*, 2013; Vera *et al.*, 2016; Prots *et al.*, 2018), and Huntington's (HD iPSC Consortium, 2012; Xu *et al.*, 2017) diseases (Table 1). These disorders exhibit altered synapse density and/or morphology as demonstrated by both animal models and postmortem patient samples (Penzes *et al.*, 2011) and are collectively referred to as synaptopathies (Brose *et al.*, 2010). However, the mechanisms that underlie synaptic alterations in the human brain are unclear.

hIPSC-derived neurons and brain organoids recapitulate human brain development in the laboratory, providing a unique opportunity to uncover the dynamic molecular events leading to altered synapse formation in neurodevelopmental disorders or synapse loss in neurodegeneration. In this review, we discuss how both hIPSC-derived two-dimensional (2D) neurons and three-dimensional (3D) brain organoids develop synapses and synaptic activity that can be used to model complex neuronal disorders.[1] Each of these models has particular strengths and challenges for simulating human synapse development and degeneration. In general, brain organoids exhibit more complexity, whereas hIPSC-derived neurons provide more uniform cultures of specific cell types (Kelava and Lancaster, 2016).

Brain organoids recapitulate many aspects of physiological human brain development. Temporally, brain organoids closely mimic human fetal brain development, as revealed by transcriptional analysis and synaptic development, thus necessitating lengthy culture times for functional synapse maturation (Lancaster *et al.*, 2013; Mariani *et al.*, 2015; Paşca *et al.*, 2015; Kelava and Lancaster, 2016). During prenatal development, excitatory synapses form along the dendrite shaft or on filopodia-like projections, but shift to specialized dendritic spines postnatally (Yuste and Bonhoeffer, 2004; Koleske, 2013). Mature dendritic spines consist of a polarized spine neck and head with an electron-dense postsynaptic density (PSD) adjacent to a presynaptic axon terminal (Lynch *et al.*, 2007). These axospinous synapses have been documented only in brain organoids (Quadrato *et al.*, 2017), although similar investigations are needed to determine whether hIPSC-derived neurons exhibit morphological changes associated with excitatory synapse formation (Marchetto *et al.*, 2010). Observing the morphological changes associated with synapse maturation suggests that organoids closely mimic human brain development.

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Abbreviations used: 2D, two-dimensional; 3D, three-dimensional; Aβ, amyloid-β; hIPSC, human-induced pluripotent stem cells; PSD, postsynaptic density.

[1]Throughout the review, hIPSC-derived neurons refer to cultures directed toward neuronal differentiation. Most hIPSC-derived neurons are 2D cultures, although some are embedded in hydrogels. Brain organoids are also derived from human IPSCs, but contain diverse neuroectodermal cells that self-assemble into 3D structures.

Author (year)	Disease	Culture time	Brain region	Synapse markers	Protocol	Key findings
Lin *et al.* (2018)	Alzheimer's disease (AD)	Up to 6 mo	Cerebrum	Excitatory: SYP PSD95	Organoid (Matrigel) Cortical neurons and astrocytes in 2D culture	• APOE4 causes AD phenotypes in neurons, astrocytes, microglia, and cortical organoids, which was partially rescued by gene editing APOE4 to APOE3. • Increased excitatory synapse formation in APOE4 neurons. • Impaired plaque clearance occurs in APOE4 astrocytes and microglia.
Prots *et al.* (2018)	Parkinson's disease	20 d of neuronal differentiation	Cortical neurons	SYN1 SNAP25 SYN38	hIPSC-derived neurons (2D)	• α-Synuclein oligomers disrupt anterograde axonal transport of mitochondria and result in synaptic degradation.
Marchetto *et al.* (2017)	Idiopathic autism	Up to 50 d	Cortical neurons	SYN1 Excitatory: vGLUT-1 Inhibitory: GABA[b]	hIPSC-derived neural progenitor cells (NPC) and neurons (2D)	• Autism increased NPC proliferation. • Autism decreased excitatory synapse formation and network activity.
Quadrato *et al.* (2017)	None	Up to 13 mo	Whole brain	Synapsin-1 (SYN1) Excitatory: vGLUT-1 Inhibitory: vGAT	Organoid (matrigel)	• Synapse maturation with age progresses similar to fetal brain development. • Excitatory synapses on spines observed by EM. • Extensive brain region diversity. • Observed spontaneous network activity and stimuli-induced activity.
Windrem *et al.* (2017)	Schizophrenia	Up to 200 d	Glial cells (astrocytes and oligodendrocytes)	Excitatory: NRXN1 NLGN1 SLITRKs 2–5 DSCAM1	hIPSC-derived glia and mouse brain chimera	• hIPSC-derived glia from schizophrenia patients result in schizophrenia-associated behaviors in mice.
Xu *et al.* (2017)	Huntington's disease (HD)	34, 48, and 50 d	Forebrain	SYP Inhibitory: GAD65 GABA[b]	hIPSC-derived neurons (2D)	• Isogenic controls rescue HD phenotypes, including impaired neural rosette formation and mitochondrial respiration and cell death.
Odawara *et al.* (2016)	None	Up to 46 wk	Cortical neurons	SYP Excitatory: PSD-95	hIPSC-derived neurons cultured on MEA plates	• hIPSC-derived neurons were maintained in culture for more than a year. • Spontaneous and evoked spiking were detected. • Clinical anticonvulsants were used to suppress epileptiform bursting.
Qian *et al.* (2016)	Zika virus-induced microcephaly	Up to 84 d	Forebrain Midbrain Hypothalamus	Excitatory: vGLUT-1 Inhibitory: GABA[b]	Organoid (spinning bioreactors)	• Zika virus decreases NPC proliferation and increases cell death.

TABLE 1: Synapses in hIPSC-derived neurons and brain organoid.

Continues

Author (year)	Disease	Culture time	Brain region	Synapse markers	Protocol	Key findings
Mariani *et al.* (2015)	Macrocephaly-associated autism spectrum disorders (ASD)	Up to 6 wk	Telencephalic (cerebrum)	SYN1 Excitatory: vGLUT-1 Inhibitory: vGAT	Organoid (free-floating)	• Increased FOXG1 and inhibitory neuron/synapse formation in macrocephaly-associated autism organoids.
Paşca *et al.* (2015)	None	Up to 9 mo	Cerebrum	SYN1 Excitatory: vGLUT1 PSD-95 NR2B	Forebrain organoid (free floating)	• Free-floating cortical spheroids form synapses and astrocytes.
Krey *et al.* (2013)	Timothy syndrome	Up to 5 wk	Cortical neurons	SYN1	hIPSC-derived neurons (2D)	• hIPSC-derived neurons model altered dendritic retraction in Timothy syndrome.
Israel *et al.* (2012)	Sporadic and familial AD	3 wk	Neurons	SYN1	hIPSC-derived neurons (2D)	• hIPSC-derived neurons recapitulate key pathological features of AD, including higher levels of amyloid-β, phospho-tau, and active glycogen synthase kinase-3β.
Shi *et al.* (2012)	None	Up to 100 d	Cortical neurons	SYP Excitatory: vGLUT-1 PSD-95 Inhibitory: Munc13-1 Homer1	hIPSC-derived neurons (2D)	• hIPSC-derived neurons model synapse formation and function.
Brennand *et al.* (2011)	Schizophrenia	Up to 6 wk	Cortical neurons	Excitatory: vGLUT-1 GAD65/67 GLUR1 PSD-95 Inhibitory: Gephyrin vGAT GABA	hIPSC-derived neurons (2D)	hIPSC-derived neurons from schizophrenia can recapitulate disease phenotypes. Loxapine rescues neuronal connectivity.

[a]This is not meant to be an exhaustive list of all hIPSC studies that examined synapses.
[b]Used primarily as a cell type marker rather than a synaptic marker.

TABLE 1: Synapses in hIPSC-derived neurons and brain organoids.[a] Continued

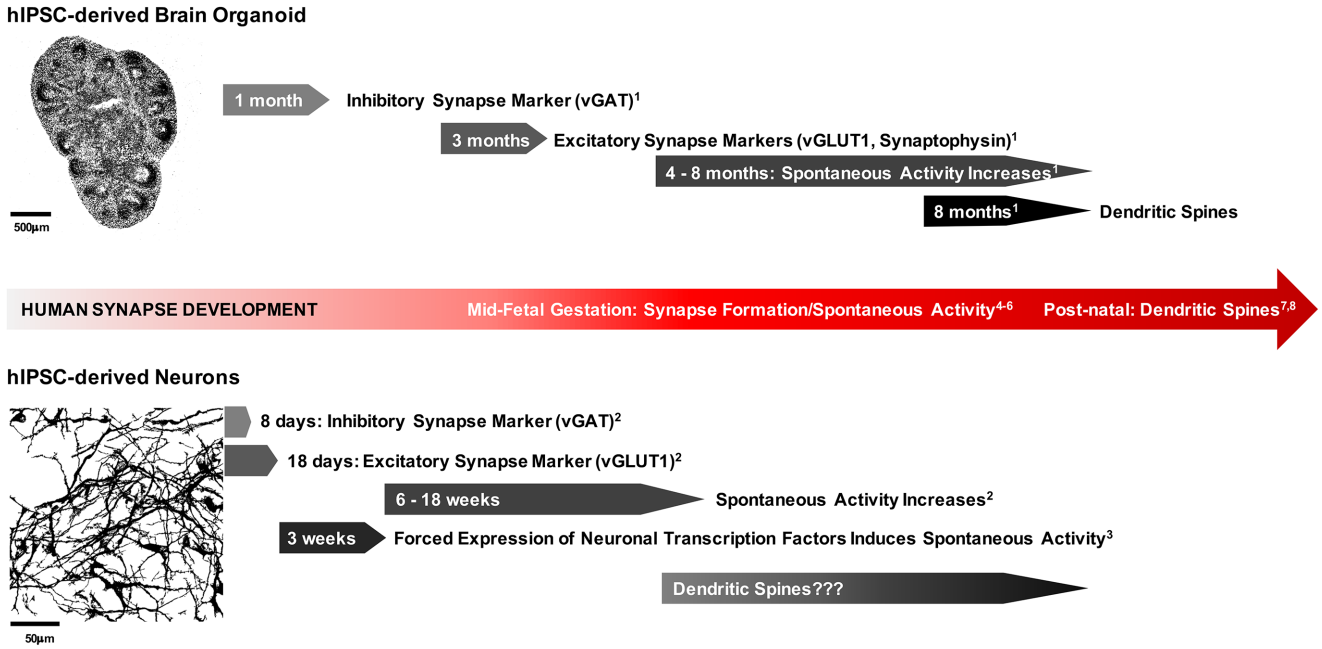

FIGURE 1: Time course of synapse development across hIPSC models. Brain organoids develop synapses and synaptic activity similar to those of the human brain. During midfetal gestation (~18 wk), synapses form and spontaneous activity begins (4–6: Tau and Peterson, 2010; Moore *et al.*, 2011; Luhmann *et al.*, 2016). Dendritic spines form postnatally (7,8: Yuste and Bonhoeffer, 2004; Koleske, 2013). In both whole-brain organoids (1: Quadrato *et al.*, 2017) and forebrain cortical spheroids (Paşca *et al.*, 2015), spontaneous activity begins after ~4 mo of culture. Furthermore, whole-brain organoids exhibit dendritic spines after 8 mo of culture (1: Quadrato *et al.*, 2017). By contrast, hIPSC-derived neurons exhibit earlier expression of synaptic markers and spontaneous activity (2: Nadadhur *et al.*, 2017) that can be increased by forced expression of neuronal transcription factors (3: Zhang *et al.*, 2013). It is unclear whether hIPSC-derived neurons form dendritic spines. The top image is a cryosection of a forebrain cortical spheroid developed according to the methods of Paşca *et al.* (2015), and the bottom image is a 2D hIPSC-derived cortical neuron culture developed according to Brennand *et al.* (2015).

In addition to modeling the time course of human brain development, brain organoids self-organize into structures that recapitulate human brain organization. They develop ventricles with surrounding progenitor zones that contain radial glia, and they form deep and superficial cortical layers (Lancaster *et al.*, 2013; Paşca *et al.*, 2015). Brain organoids also contain astrocytes (Lancaster *et al.*, 2013; Paşca *et al.*, 2015; Dezonne *et al.*, 2017; Quadrato *et al.*, 2017; Sloan *et al.*, 2017). Astrocytes critically support synapse development, maintenance, and function, and are thus important to consider when modeling synaptic disorders (Allen and Eroglu, 2017). In certain cases, myelinating oligodendrocytes have also been described in brain organoids (Di Lullo and Kriegstein, 2017; Madhavan *et al.*, 2018), although most myelination occurs postnatally (Miller *et al.*, 2012). Organoids also recapitulate biophysical properties of human brain development, allowing for events such as cortical folding (Li *et al.*, 2017; Karzbrun *et al.*, 2018). The 3D matrix of organoids may also retain secreted factors that regulate synaptic development and degeneration, as suggested by the use of hydrogels to capture amyloid plaques in Alzheimer's human neural cell culture (Choi *et al.*, 2014, 2016). Thus, brain organoids capture the cellular diversity, temporal dynamics, and structural organization of the human brain.

However, depending on the protocol, organoids contain diverse ratios of particular cell types. For example, whole-brain organoids contain hindbrain, midbrain, forebrain, and even retinal cells (Kelava and Lancaster, 2016; Quadrato *et al.*, 2017), whereas hIPSC-derived neurons can be directed toward specific lineages and/or enriched by fluorescence-analyzed cell sorting. Addition of astrocytes to neuronal cultures promotes synapse maturation and spontaneous action potential formation. This can be accomplished through 1) cultures that generate both neurons and astrocytes through a shared progenitor, 2) hIPSC-derived neuron-astrocyte cocultures, or 3) application of astrocyte-conditioned media to hIPSC-derived neurons (Tang *et al.*, 2013; Odawara *et al.*, 2016; Gunhanlar *et al.*, 2018; Xie *et al.*, 2018). Defined cocultures can also be used to address how specific cell types contribute to disease progression. For example, Down syndrome hIPSC-derived astrocytes compromise synaptogenesis of unaffected hIPSC-derived neurons (Araujo *et al.*, 2018). Furthermore, forced expression of neuronal transcription factors, either neurogenin-2 or neuroD1, can rapidly induce neuronal maturation and functional synaptic activity within 3 wk of hIPSC-derived neuron culture (Zhang *et al.*, 2013). Thus, while organoids enable the study of synapses in a complex environment that mimics human fetal brain development, hIPSC-derived neurons allow for the enrichment of specific brain cell types and rapid induction of physiological maturation (Figure 1).

In this review, we address the ability of both hIPSC-derived neurons and brain organoids to recreate altered synapse development and degeneration in the lab,[2] which will ultimately enable therapeutic screens for restored synaptic connections. We also discuss opportunities for improved hIPSC models that more accurately reflect human synaptopathies.

[2]Unless otherwise noted, the following sections focus on hIPSC-derived 3D whole-brain organoids and 2D forebrain cortical neurons (Muratore *et al.*, 2014; Brennand *et al.*, 2015; Griesi-Oliveira *et al.*, 2015; Kelava and Lancaster, 2016).

SYNAPSE DEVELOPMENT

Before hIPSC-derived models were available, research on human synapses was limited to a static snapshot of brain development available from postmortem tissue. Postmortem studies predominantly focused on excitatory synapses at dendritic spines, which are evident by Golgi staining (Mancuso *et al.*, 2013). Dendritic spines are the primary sites of excitatory stimulation leading to long-term potentiation and learning and memory formation (Lynch *et al.*, 2007). In an immature state, excitatory synapses form between the presynaptic axon and the dendritic shaft or filopodia-like spine precursors (Yuste and Bonhoeffer, 2004). Spines then mature into a polarized mushroom-shaped structure with a distinct head and neck (Yuste and Bonhoeffer, 2004). Glutamate receptors cluster at the tip of the spine head in the electron-dense PSD (Lynch *et al.*, 2007). In the human prefrontal cortex, excitatory synaptogenesis begins around 18 wk of fetal gestation and coincides with spontaneous action potential formation (Tau and Peterson, 2010; Moore *et al.*, 2011). Early synaptogenesis occurs primarily along the dendritic shaft, but shifts to dendritic spines in later peri- and postnatal development (Yuste and Bonhoeffer, 2004; Petanjek *et al.*, 2011). Excitatory synapses are then either strengthened or pruned in an activity-dependent manner throughout childhood, leading to relatively stable synapses in adulthood (Penzes *et al.*, 2011). Conversely, inhibitory synapses suppress action potential formation and refine the amount of excitatory synapses (Craig and Kang, 2007). Inhibitory synapses form almost exclusively along the dendritic shaft and lack the electron-dense PSD and are thus not evident by Golgi staining or electron microscopy without the use of immunogold labeling. Despite difficulties visualizing inhibitory synapses in postmortem tissue, it is known that they critically regulate brain development and cognitive function. Disruptions in the ratio of excitatory to inhibitory synapses contribute to both neurodevelopmental and neurodegenerative disorders (Penzes *et al.*, 2011; Gao and Penzes, 2015).

ALTERED SYNAPSE FORMATION IN NEURODEVELOPMENTAL DISORDERS

Golgi staining of autism postmortem brain tissue reveals increased dendritic spines on cortical pyramidal neurons (Hutsler and Zhang, 2010; Tang *et al.*, 2014). The observed increase in dendritic spines, the primary sites of excitatory neurotransmission, suggests increased excitatory-to-inhibitory signaling in autism (Takarae and Sweeney, 2017). This hyperexcitability could account for the increased risk of epilepsy in the autism population (Tuchman and Rapin, 2002). However, electroencephalographic data from living patients suggest a more complex picture than global hyperexcitability, as autism patient brains exhibit local areas of hyperexcitability balanced by underconnectivity at longer distances (Boutros *et al.*, 2015). It is also unclear whether the increased number of synapses in autism are functional and stabilized into mature synaptic connections or exist as silent synapses without activity. Silent synapses may be subject to greater loss, leading to neurodegeneration and regression in autism (Hanse *et al.*, 2013; Kern *et al.*, 2013). It is critical that human brain models capture the lifetime of synapse formation, refinement, maintenance, and loss to accurately reflect disease progression.

Unlike in autism, schizophrenia postmortem brains exhibit decreased dendritic spines in both the cortex and hippocampus (Glantz and Lewis, 2000; Law *et al.*, 2004; Kolomeets *et al.*, 2005; Lewis and Sweet, 2009; Sweet *et al.*, 2009). However, decreased GABAergic inhibitory signaling in schizophrenia may lead to a hyperexcitable state, similar to autism (Gao and Penzes, 2015). This further highlights the need for human brain models that capture the altered development of both excitatory and inhibitory synapses to unravel the complexities of neurodevelopmental disorders.

STEM CELL MODELS OF SYNAPSE DEVELOPMENT

hIPSC-derived neurons and brain organoids demonstrate a remarkable ability to mirror human fetal synapse development and provide insights into neurodevelopmental pathologies. In both models, excitatory synapse formation and spontaneous action potentials increase with age similar to the time course of prenatal brain development (Shi *et al.*, 2012; Odawara *et al.*, 2016; Quadrato *et al.*, 2017) (Figure 1). By 8 mo in culture, brain organoids exhibit excitatory synapses on dendritic spines; reconstruction of serial-section electron microscopy reveals that 30 out of 37 synapses formed on dendritic spines (Quadrato *et al.*, 2017). By contrast, after 300 d of hIPSC-derived neuron coculture with astrocytes, excitatory synapses still primarily form along dendrites and soma (Odawara *et al.*, 2016). Notably, the number of synaptophysin-positive excitatory synapses more than doubled between 112 and 300 d of neuron–astrocyte coculture (Odawara *et al.*, 2016). In both hIPSC-derived neurons and brain organoids, spontaneous action potentials (spikes) increased with culture time. Spontaneous action potentials begin early in development in the absence of a stimulus and play important roles in establishing neural circuits (Luhmann *et al.*, 2016). At 8 mo, brain organoid spike frequency varied from 0 to 6 Hz (mean = 0.66 Hz) (Quadrato *et al.*, 2017). By contrast, hIPSC-derived neurons cocultured with astrocytes averaged a spike frequency of ~2 Hz at 6 wk, which increased to ~5 Hz between 12 and 18 wk and remained constant through 34 wk of culture (Odawara *et al.*, 2016). Forced expression of neuronal transcription factors, either neurogenin-2 or NeuroD1, can be used to accelerate synapse function, resulting in spontaneous action potentials after 3 wk of culture (Zhang *et al.*, 2013). Thus, hIPSC-derived neurons acquire functional synapses earlier than brain organoids, and this activity can be accelerated through forced expression of neuronal-specific genes.

hIPSC-derived neurons and brain organoids form specialized excitatory and inhibitory synapses. In brain organoids, excitatory synapse markers, such as synaptophysin-1 (syn-1) and vesicular glutamate transporter-1 (vGLUT-1), express after 3 mo of culture, while the inhibitory presynaptic marker, vesicular GABA Transporter (vGAT), expresses at 1 mo of culture (Quadrato *et al.*, 2017). Similarly, in hIPSC-derived neurons, vGAT expression increases at 8 d of culture, preceding increased vGLUT-1 expression after 18 d (Nadadhur *et al.*, 2017). vGAT expression before glutamatergic synapse expression likely reflects the early developmental phenomenon of GABA-induced excitation. GABA-induced excitation mediates neuronal migration and the formation of neuronal connections, while preventing glutamate cytotoxicity in early development (Ben-Ari and Tyzio, 2009; Bortone and Polleux, 2009). Later in prenatal development, GABA switches from excitation to inhibition through expression of the potassium chloride transporter KCC2, thus establishing an excitatory-to-inhibitory balance (Bortone and Polleux, 2009; Ben-Ari, 2014; Sivakumaran *et al.*, 2015; Leonzino *et al.*, 2016). Forebrain organoids exhibit GABA-induced excitation and show increased KCC2 expression with longer development (84 wk) (Qian *et al.*, 2016). By contrast, GABA-induced excitation decreases after 2 wk of hIPSC-derived neuron culture (Rushton *et al.*, 2013), providing further support that brain organoids develop similarly to the brain in utero, while hIPSC-derived neurons exhibit accelerated synapse formation and function.

STEM CELL MODELS OF SYNAPTIC ALTERATIONS IN NEURODEVELOPMENTAL DISORDERS

In addition to recapitulating normal synapse formation and function, hIPSC brain models provide insights into the molecular mechanisms underlying synaptic abnormalities in neurodevelopmental disorders. Both schizophrenia and autism exhibit synaptic abnormalities in the cerebral cortex (Hutsler and Zhang, 2010; Selemon and Zecevic, 2015; Ajram *et al.*, 2017). hIPSC-derived neurons from schizophrenia patients exhibit reduced neuronal connections and decreased RNA expression of excitatory synapse markers, including PSD-95 and glutamate receptors, which are rescued by the antipsychotic drug Loxapine (Brennand *et al.*, 2011). In a study of autism patients with larger than average head size, hIPSC-derived neurons exhibit decreased excitatory synapses and action potentials and increased inhibitory synapse markers (Marchetto *et al.*, 2017). Similarly, macrocephaly-associated autism forebrain organoids form more inhibitory neurons and synapses (Mariani *et al.*, 2015). These results challenge the simplified notion of hyperexcitability in autism, particularly for macrocephaly-associated patients. Similarly, hIPSC-derived neurons from autism patients with Rett syndrome exhibit decreased excitatory synapses (Marchetto *et al.*, 2010) but increased GABA-induced excitation, which is rescued by KCC2 expression (Tang *et al.*, 2016). These results demonstrate how hIPSC-derived neurons can reveal patient-specific differences in genetically diverse spectrum disorders.

In addition to modeling genetic causes of altered synapse development, hIPSC-derived neuronal models can be used to examine the impact of environmental factors on neurodevelopment. For example, the hormone oxytocin promotes KCC2 expression and the shift from GABA-induced excitation to inhibition (Leonzino *et al.*, 2016). Autism impairs the oxytocin-mediated GABA shift (Ben-Ari, 2014). Thus, hIPSC brain organoids have the potential to model hormone action at the level of synapses and synaptic function. This particular feature will be important for addressing how environmental factors that disrupt hormonal balances contribute to neurodevelopmental disorders (Moosa *et al.*, 2018), as well as the potential benefits of hormone therapies (DeMayo *et al.*, 2017).

FUTURE ADVANCES IN STEM CELL MODELS OF SYNAPSE DEVELOPMENT

Although hIPSC-derived brain models reflect many aspects of human synapse formation and function, there are opportunities for improvement in the areas of synapse development and the modeling of neurodevelopmental disorders. One major limitation is the heterogeneity of cell types in brain organoids. In matrigel-embedded whole-brain organoids, only 30% of organoids express forebrain markers (Quadrato *et al.*, 2017). Because autism and schizophrenia primarily affect forebrain regions, whole-brain organoids may not be best suited to model certain disorders. Using the matrigel-embedding protocol, it will be necessary to screen and select for specific brain regions to compare synapse development in patients and typically developing individuals. Alternatively, more recent protocols drive neuronal identity toward specific brain regions, such as forebrain cortical spheroids (Paşca *et al.*, 2015) or brain region–specific organoids cultured in miniature bioreactors (Qian *et al.*, 2016, 2018). Furthermore, while brain organoids exhibit spontaneous network activity, the ability to recreate activity-dependent postnatal synaptic refinement is needed to address how synapse strengthening and/or pruning is altered in neurodevelopmental disorders (Penzes *et al.*, 2011). Recent research demonstrates that the activity of photoreceptor cells in brain organoids can be modulated with light after 7–9 mo in culture, demonstrating the potential to study activity-dependent synaptic refinement (Quadrato *et al.*, 2017). Additionally, optogenetics can be used to control local and global networks of synaptic activity in hIPSC-derived neuronal models (Klapper *et al.*, 2017). Finally, the recent engraftment of human brain organoids into mouse brains (Mansour *et al.*, 2018) provides a model to assess how neurodevelopmental disorders affect synapse formation and refinement in vivo. hIPSC–animal chimeras may also be used to address how altered synapse formation affects behavior, similar to research in which schizophrenia hIPSC-derived glial cells engrafted into the mouse brain resulted in schizophrenia-like behaviors (Windrem *et al.*, 2017).

STEM CELL MODELS OF NEURODEGENERATIVE DISEASE

While hIPSC-derived neuronal models reflect neurodevelopmental pathology, their developmental stages, which are similar to those of the human fetal brain, present a challenge for modeling synapse loss in neurodegenerative disorders. hIPSC research shows promise for overcoming this challenge and providing insights into the mechanisms of synapse loss in neurodegenerative disorders. Synapse loss precedes neurodegeneration in Alzheimer's, Parkinson's, and Huntington's diseases (Wishart *et al.*, 2006; Koffie *et al.*, 2011; Bellucci *et al.*, 2016). Researchers need experimental models to observe and manipulate the dynamic molecular events underlying synapse loss, especially because animal neurodegenerative models can lack key pathological features. For example, mouse models of familial Alzheimer's disease lack tau neurofibrillary tangles (Choi *et al.*, 2014). Thus, brain models are needed to capture human-specific neurodegenerative pathology. These models will allow us to address how synapse loss contributes to neurodegeneration and to develop therapies for synapse recovery.

In Alzheimer's disease, neurofibrillary tangles first emerge in the cortex and then spread to the hippocampus (Serrano-Pozo *et al.*, 2011). hIPSC-derived cortical neurons from patients with familial and sporadic Alzheimer's disease exhibit increased amyloid-β (Aβ) deposition and phosphorylated tau; however, no differences in the density of synapsin-1–positive synapses was observed, and cell death was not examined (Israel *et al.*, 2012). In a recent study of hIPSC-derived neurons, astrocytes, and microglia-like cells containing the Alzheimer's disease–associated APOE4 variant, neurons exhibited increased excitatory synapses, while astrocytes and microglia showed impaired Aβ clearance (Lin *et al.*, 2018). Alzheimer's disease hIPSC-derived neurons may require enhanced aging techniques or insults that trigger neuronal cell death in order to study synapse loss in neurodegeneration. For example, growth factor withdrawal increases cell death in a Huntington's disease hIPSC model of cortical neurodegeneration (Estrada-Sánchez and Rebec, 2013); however, the effect on synapses was not examined (Xu *et al.*, 2017). In another example of insult promoting neurodegenerative pathology, α-synuclein oligomers impair axonal transport leading to synaptic degeneration in Parkinson's disease hIPSC-derived cortical neurons (Prots *et al.*, 2018). While the cortex is affected in Parkinson's disease, the midbrain, particularly the substantia nigra, is the primary site of neurodegeneration (Maiti *et al.*, 2017). Telomerase inhibition or progerin-mediated aging of Parkinson's disease hIPSC-derived midbrain dopaminergic neurons induces DNA damage, mitochondrial oxidative stress, and dendrite degeneration, similar to early stages of Parkinson's disease (Miller *et al.*, 2013; Vera *et al.*, 2016). The use of telomerase inhibition or progerin to promote aging-related telomere shortening suggests that aging techniques and genetic susceptibility are both necessary for using hIPSCs to model neurogenerative disorders.

ADVANCES IN STEM CELL MODELS OF SYNAPSE DEGENERATION

To fully illuminate the mechanisms of synapse loss in neurodegeneration, hIPSC models need to incorporate microglia, the brain's resident immune cells. Microglia mediate synapse loss in Alzheimer's disease (Hansen *et al.*, 2017). Because microglia derive from mesodermal lineages, rather than neuroectodermal lineages, they have been largely overlooked in hIPSC-derived neuronal models (Chan *et al.*, 2007). However, recent protocols provide instructions for developing microglia-like cells from hIPSCs (Abud *et al.*, 2017; Pandya *et al.*, 2017), allowing researchers to interrogate how microglia shape human synapse formation and loss. Chimeras of patient and unaffected control microglia and neurons will help to determine the specific contribution of microglia to disease pathology.

Furthermore, transdifferentiation of patient fibroblasts into neurons, rather than reprogramming through a stem cell intermediate, allows for the preservation of age-related epigenetic profiles and shortened telomeres that may more accurately capture neurodegenerative pathology (Victor *et al.*, 2018). Transdifferentiated medium spiny neurons from adult Huntington's disease patients exhibit huntingtin (HTT) aggregates, DNA damage, mitochondrial dysfunction, and neurodegeneration (Victor *et al.*, 2018). While transdifferentiated neurons can be engineered into specific neuronal populations, this limited fate currently prevents brain organoid generation from transdifferentiation protocols.

CONCLUDING REMARKS

Through the use of hIPSC brain models, we can begin to address the fundamental molecular and cellular mechanisms underlying human neurodevelopment and neurodegeneration. Recent advances in hIPSC-derived brain organoids, such as cell-type and brain region–specific protocols, optogenetics, aging protocols, and hIPSC–mouse chimeric models will provide even greater insights into the mechanisms of synapse formation and loss. As hIPSC-derived neurons continue to better model brain disorders, they will be powerful tools for drug development and assessment of patient-specific responses to drug therapies.

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