The iPSC perspective on schizophrenia

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Feature Review

The iPSC perspective on schizophrenia

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Over a decade of schizophrenia research using human induced pluripotent stem cell (iPSC)-derived neural models has provided substantial data describing neurobiological characteristics of the disorder *in vitro*. Simultaneously, translation of the results into general mechanistic concepts underlying schizophrenia pathophysiology has been trailing behind. Given that modeling brain function using cell cultures is challenging, the gap between the *in vitro* models and schizophrenia as a clinical disorder has remained wide. In this review, we highlight reproducible findings and emerging trends in recent schizophrenia-related iPSC studies. We illuminate the relevance of the results in the context of human brain development, with a focus on processes coinciding with critical developmental periods for schizophrenia.

Highlights

Over a decade of schizophrenia research using human iPSC-based neuronal models has enhanced our understanding of the neurobiological characteristics of the disorder.

Studies using iPSC-based models of schizophrenia have identified alterations in neural progenitor cell proliferation, imbalanced differentiation of excitatory and inhibitory cortical neurons, and failure to establish projection neuron subpopulations. Many of these impairments have been associated with altered WNT signaling during neurogenesis.

Studies in neurons derived from patients with schizophrenia have identified alterations in both excitatory and inhibitory neurotransmission, as well as disrupted synaptic maturation.

iPSC-based models have shed light on the contribution of genetic risk factors and prenatal environmental insults to the development of schizophrenia and other psychiatric disorders.

Schizophrenia from a developmental perspective

Risk genes of schizophrenia and other mental disorders are highly expressed in the human brain during midgestation, a time period coinciding with cortical neurogenesis, axonal pathfinding, and neuronal functional development [1–5]. In turn, the typical onset age of schizophrenia is from the early 20s to 30s [6,7], and is preceded by cortical excitatory synaptic remodeling and maturation of the inhibitory system [8,9]. Neuroimaging and postmortem studies have detected cortical layer disorganization [10] and loss of specific neuronal subtypes [11] in the brains of patients with psychiatric disorders. However, the origin of these changes has remained elusive. Likewise, altered structural and functional brain connectivity have been linked to schizophrenia but the neurobiological mechanisms driving these changes are not fully understood [12–19].

The developmental timeline of iPSC-derived neurons in culture recapitulates certain aspects of fetal brain development [2,20,21] and, together with the ability to incorporate genetic risk variants into these models, iPSC-derived brain cells have provided a valuable tool for studying neurobiological characteristics of schizophrenia. Based on evidence from iPSC studies, differences in brain development in health and schizophrenia appear to arise during neurogenesis [2,22,23]. Aberrant neural progenitor cell (NPC) proliferation and differentiation into neurons have been linked to changes in cortical morphogenesis and cell type composition in patient-specific neuronal models [22,23]. In addition, alterations in neuronal function, including excitatory–inhibitory imbalance, have been detected in iPSC-based models of schizophrenia [24,25].

In this review, we discuss findings from iPSC studies of schizophrenia and emerging trends in the field. The findings are arranged under three main topics: cortical neurogenesis, brain connectivity, and brain functional development. Each of the three sections is accompanied by an introductory box (Boxes 1–3) that summarizes important aspects of the relevant developmental process *in vivo* and *in vitro*. The boxes offer a context for the subsequent discussion of schizophrenia-related abnormalities found in iPSC models of schizophrenia. Figure 1 (Key figure) presents a timeline of the developmental phenomena discussed throughout the article.

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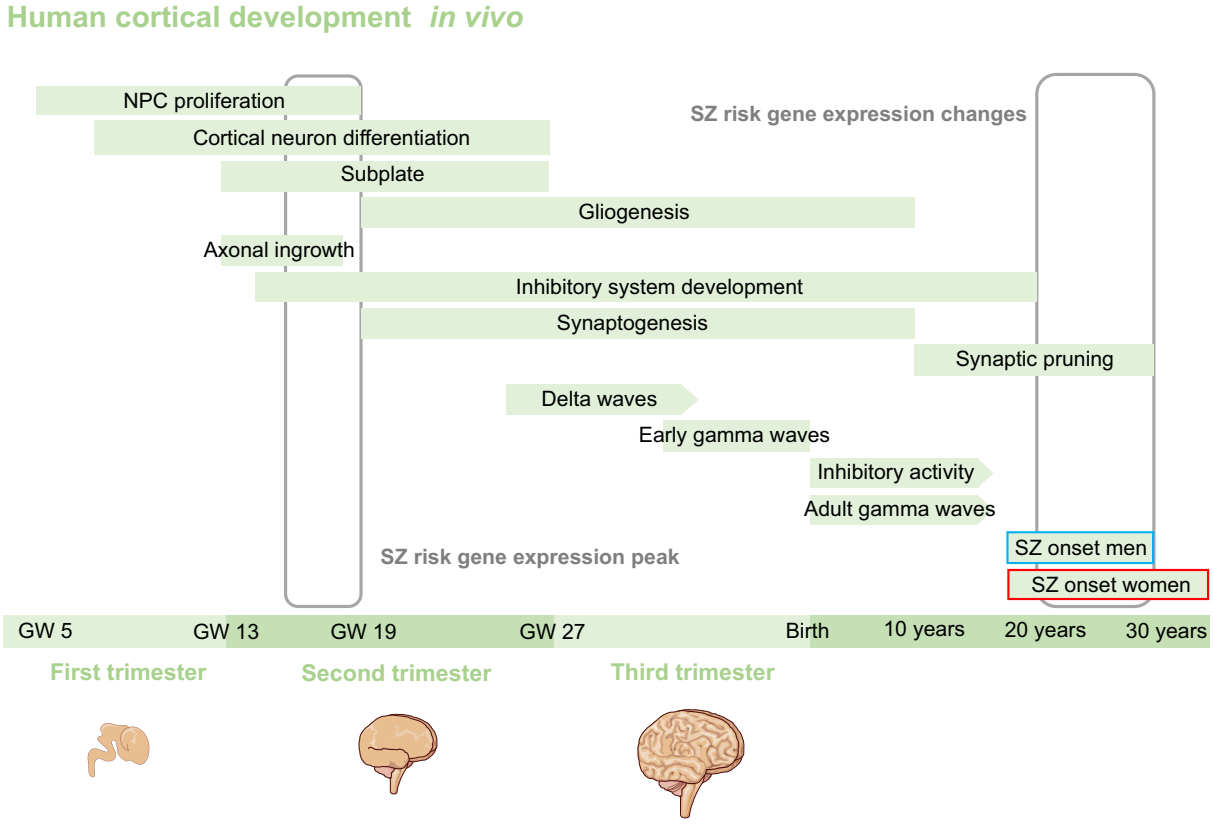

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Box 1. Cortical neurogenesis *in vivo* and *in vitro*

Cortical neurogenesis in the human brain starts at GW5 by generation of NPCs in the ventricular zones of the cortical wall [34,35]. Corticogenesis starts when reelin-expressing interneurons migrate to the cortex and form the first cortical layer. The reelin-secreting neurons guide the entrance of glutamatergic neurons to the cortical plate starting from GW 7–8 [35]. Simultaneously, interneurons from the ganglionic eminence migrate toward the cortex and innervate the cortical plate alongside locally differentiated glutamatergic neurons [36]. The early-born glutamatergic neurons form the deep layers of the neocortex during the first trimester [37]. At the beginning of the second trimester, a new progenitor zone, called the outer subventricular zone, appears and expands massively over the following month. The progenitor cells in this new zone differentiate into both glutamatergic and GABAergic neurons [34]. Simultaneously, a new cortical region, called the subplate, emerges between the progenitor zones and the cortical plate. The subplate harbors postmitotic neurons during the second trimester, before the neurons enter the cortical plate [3,38]. The superficial cortical neurons differentiate during the second trimester and complete the genesis of a six-layered neocortex [37].

Using human iPSCs, cortical neurons can be differentiated *in vitro* in a time-dependent manner corresponding to neurogenesis *in vivo* [20]. By traditional, directed differentiation, the generation of NPCs is induced in dual SMAD inhibition by blocking the BMP and TGF-β signaling pathways [20]. In a well-established differentiation protocol [20], TBR1-expressing layer VI neurons and CTIP2-expressing layer V neurons appear within days after neural induction and are mostly differentiated by days 30 and 35. Superficial layer BRN2-expressing layer II/III neurons are mostly generated by day 45 and are followed by SATB2-expressing neurons between days 65 and 80 [20]. Importantly, the iPSC-derived neuronal cultures correspond to the early and mid-fetal periods of brain development based on their gene expression patterns [2,39]. In addition to the conventional, directed differentiation, a method using forced expression of the NGN2 transcription factor, to obtain excitatory neurons with features of superficial layer cortical neurons, has gained popularity in recent years [40,41]. In addition to the protocols yielding mainly excitatory neurons, cortical interneurons can be obtained by directed differentiation or by induced expression of ASCL1 and DLX2 transcription factors [42,43].

Cortical neurogenesis in schizophrenia

Cortical neurogenesis is a vulnerable time for brain development. The rate of gene expression changes has been estimated to be more than 100 times faster than in the adult brain [26], genes intolerant to mutations are mostly expressed during midpregnancy [27], and environmental insults during this period have been linked to aberrant brain development and risk for compromised mental health later in life [28,29]. Not surprisingly, mental disorder-associated risk genes are highly expressed during midgestation. Genes associated with schizophrenia, autism spectrum disorder (ASD), and major depression (MD) share similarities in their prenatal expression trajectory, with a peak at 16–19 gestational weeks (GW). Many risk genes for bipolar disorder (BP) reach their expression peak shortly after, at 19–22 GW. In schizophrenia, prenatally expressed risk genes are often associated with cell fate specification and morphogenesis [1,30]. Postmortem and brain-imaging studies of schizophrenia have found abnormalities in cortical cell-type composition and macroscopic tissue organization, possibly stemming from aberrant brain development. Among the most prominent alterations reported in these studies are a reduced density of parvalbumin (PV)-expressing interneurons in the prefrontal cortex (PFC) [11], decreased thickness of the superficial cortical layers [31,32], and increased lateral ventricle volume [33] (Box 1).

Alterations in WNT signaling

Using patient-derived and genetically edited iPSC lines, several studies have found alterations in cortical neurogenesis linked to schizophrenia. In an increasing number of studies, these abnormalities have been associated with altered expression of WNT signaling pathway components, including TCF/LEF transcription factors and GSK3 [22,44–50]. An early iPSC study that investigated the consequences of WNT signaling abnormalities in schizophrenia used NPCs with engineered DISC1 exon 2/8 interruption [46], a rare strong genetic variant for schizophrenia, BP, and MD. The affected

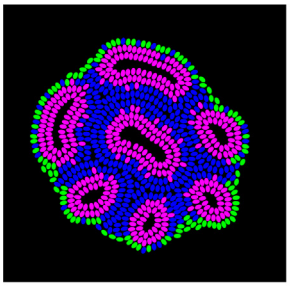
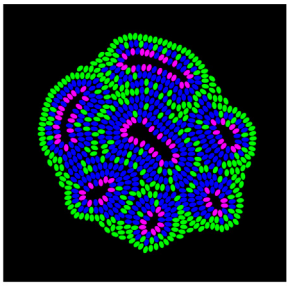
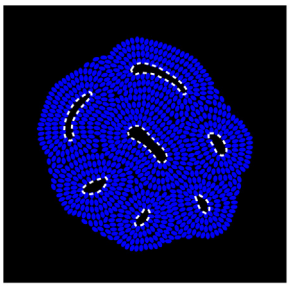
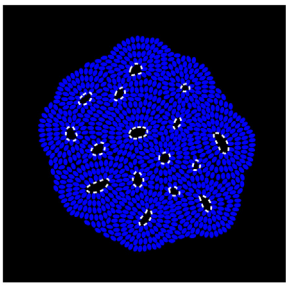
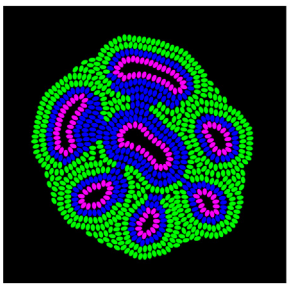
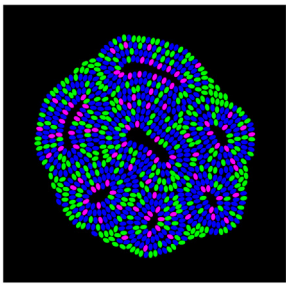
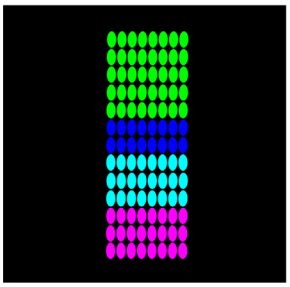
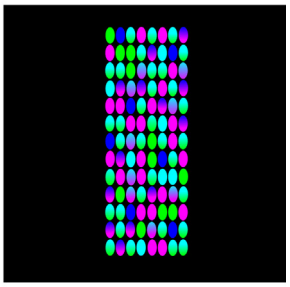
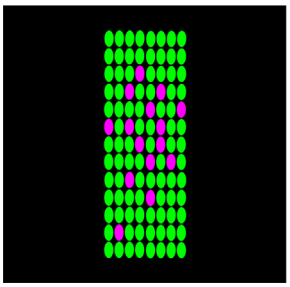
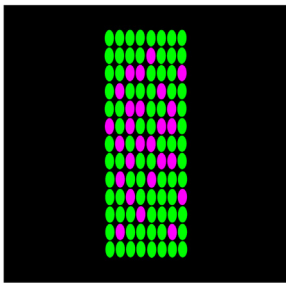
Figure 1. Timeline of human brain development *in vivo* and *in vitro*. The timeline presents critical developmental periods for schizophrenia (SZ) and related developmental processes *in vivo*. SZ risk genes are highly expressed during midgestation, coinciding with the differentiation of cortical neurons, subcortical afferent ingrowth to the cortex, and the beginning of synaptic development. The onset of SZ coincides with a period of heightened synaptic pruning, which is preceded by maturation of the inhibitory system. Human iPSC-based models recapitulate aspects of fetal brain development *in vitro*. The functional maturation of cortical neurons requires interplay between excitatory neurons, inhibitory neurons, and glial cells. Abbreviations: GW, gestational week; NPC, neural progenitor cell.

NPCs were found to exhibit elevated WNT signaling activity accompanied by altered expression of neuronal fate-related genes, including increased expression of dorsal progenitor markers and decreased expression of ventral progenitor markers. When the affected NPCs were treated with a WNT antagonist, the phenotype was rescued [46]. DISC1 mutation in exon 8 was later found to cause disorganization of ventricular structures, decreased NPC proliferation, and reduced expression of BRN2 in the superficial layer neurons in an organoid model (Figure 2) [47]. The alterations were again rescued with a WNT antagonist [47]. Importantly, the effect of altered Wnt/β-catenin signaling on cortical morphogenesis has been illuminated in rodent studies, with some of the outcomes similar to those obtained using the iPSC models of DISC1 mutation. Specifically, overexpression of β-catenin during corticogenesis has been shown to cause: (i) overproduction of ventricular zone progenitors and deep-layer projection neurons; (ii) underproduction of subventricular zone progenitors and superficial-layer neurons; and (iii) ventricular enlargement [51,52]. Mouse models have also provided mechanistic insights into the role of Disc1 as a regulator of WNT signaling activity by revealing direct physical interactions between Disc1 and the WNT signaling mediator GSK3β [53]. In addition to the increased expression of WNT signaling components observed in iPSC models with edited DISC1 mutations, such increases have been detected in neurons derived from patients with schizophrenia [25,45].

In contrast to the findings from the DISC1 mutation models, decreased WNT signaling activity was recently detected in iPSC-derived brain organoids from patients with schizoaffective disorder and schizophrenia [22]. The reduced WNT signaling activity was accompanied by enhanced GABAergic neuron differentiation, reduced NPC proliferation, and accelerated neuronal maturation (Figure 2). In monolayer cultures, the patient-derived neurons were found to contain an increased number of inhibitory neurons and elevated inhibitory synaptic density after 120 days of differentiation. Here, activation of WNT signaling before neuronal maturation normalized the number of GABAergic neurons [22]. In line with this study, elevated ventral neuronal gene expression and altered expression of WNT pathway components have been found in iPSC-derived neurons of patients with BP or ASD [54–56]. Altogether, these results imply that alterations in NPC proliferation and excitatory–inhibitory neuronal differentiation in schizophrenia and associated disorders may arise during cortical neurogenesis due to altered WNT signaling. However, in the developing brain, excitatory and inhibitory neurons are generated in separate brain regions (Box 1) that are not recapitulated in brain organoids or monolayer cultures. The genesis and migration of excitatory and inhibitory neurons during corticogenesis could be modeled more accurately using fused cortical and ventrally specified organoids [57].

Prenatal immune activation

The heritability of schizophrenia is as high as 79%, whereas the concordance of the disorder in monozygotic twins is only 33%, indicating that interactions between genetic and environmental risk factors have a substantial role in the development of the disorder [58]. Among the factors that might contribute to brain maldevelopment in schizophrenia are maternal immune activation (MIA) and stressful life events during midpregnancy [28,29]. The effects of MIA on cortical development have been studied extensively using animal models, although some of the specifics continue to be debated. In mice, MIA has been shown to result in abnormal neuronal proliferation, radial migration, and cell type composition in the cortex. These alterations have long-lasting effects on brain functional maturation and animal behavior [29,59,60]. Cortical GABAergic neurons reportedly exhibit specific vulnerability to environmental insults [59,61]. MIA has been shown to affect interneuron proliferation, with early insults [embryonic day (E) 9.5] reducing proliferation and late insults (E16.5) increasing proliferation [59]. In addition, MIA has been shown to impair the functional development of PV-expressing interneurons in the mouse PFC [61].

Feature description	Healthy control	Schizophrenia
Decreased NPC proliferation (magenta) and accelerated neuronal differentiation (green) [22,23,47]		
Decreased size and increased number of ventricular structures [47]		
Displacement of proliferating cells (magenta) into cortical plate and post-mitotic neurons (green) into progenitor zones [23]		
Postmitotic neurons express multiple cortical layer-specific transcription factors and fail to settle into correct cortical layers [104]		
Increased or decreased differentiation of glutamatergic (green) or GABAergic (magenta) neurons [22,47]		

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The impact of prenatal immune activation on cortical development was addressed in iPSC studies only recently [62–64]. In one of these studies, IFN-γ treatment was found to partially recapitulate transcriptomic changes typical for schizophrenia and ASD in healthy iPSC-derived NPCs and neurons [62]. NPCs exposed to IFN-γ showed long-lasting upregulation of genes in the major histocompatibility complex (MHC) I region and general dysregulation of genes overlapping with schizophrenia risk genes, including synapse-related genes [62]. These findings imply that early environmental insults may indeed trigger expression changes in genes associated with the development of psychiatric disorders. In another study, iPSC-derived interneurons from patients with schizophrenia and healthy controls were cultured with activated microglia-conditioned medium [64]. After the treatment, the patient-derived neurons exhibited long-lasting metabolic dysfunction and reduced GABA release, whereas the control neurons recovered after an acute response. When glutamatergic neurons were exposed to the same treatment, neither patient-derived nor control neurons showed metabolic deficits [64]. The finding provides supporting evidence for the observation that cortical interneurons are sensitive to prenatal immune insults [59,61]. Notably, the long-lasting cellular dysfunction was induced only through interplay between intrinsic risk factors and environmental insults. It was also recently shown that astrocytes derived from patients with schizophrenia had a flattened response to proinflammatory cytokine IL-1β and a reduced ability to recruit regulatory T cells in a migration assay compared with control astrocytes [65]. Altogether, these findings suggest that a reduced ability to react to immune insults and recover from them underlies vulnerability to developing a psychiatric disorder. However, the role of environmental insults in schizophrenia has been substantially less studied with iPSC-derived brain cells compared with genetic risk factors, and models investigating the cooperative effect of these two risk factors on the disease phenotype are still largely missing.

ECM abnormalities

An increasing number of iPSC studies have observed dysregulation of extracellular matrix (ECM)-related pathways and processes in schizophrenia [48,49,63,66–70]. Two studies identified the hepatic fibrosis/hepatic stellate cell activation pathway enriched with collagen genes as the top dysregulated canonical pathway in patient-derived neurons [66,70]. Similar results have been obtained in a preliminary transcriptomic analysis of patient-derived astrocytes [50], the brain equivalent of ECM-producing hepatic stellate cells [71]. Recently, major abnormalities in collagen gene expression were also found in patient-derived interneurons [69]. Notably, accumulation of fibrous ECM has been detected in embryonic stem cell-derived organoids after exposure to TNF-α [63], indicating that ECM remodeling in the brain may occur as a consequence of an inflammatory response. When investigating organoids derived from a patient with schizophrenia, comparable fibrous ECM accumulation was observed. The ECM accumulation in TNF-α-treated organoids and patient-derived organoids was accompanied by dispersion of proliferating cells into the cortical plate and cell clustering within scar-like ECM [63]. In an earlier study by the same group [23], similar cortical disorganization accompanied by reduced expression of the ECM protein reelin was detected in organoids derived from iPSCs of three patients with schizophrenia (Figure 2). Altogether, these findings provide robust evidence for broad ECM-related abnormalities in schizophrenia, possibly stemming from a cellular stress response and leading to abnormal cortical morphogenesis.

Figure 2. Alterations in induced pluripotent stem cell (iPSC)-derived brain organoids modeling schizophrenia. Differences in the development of brain cells in health and schizophrenia have already emerged during neurogenesis. Several iPSC studies have reported decreased neural progenitor cell (NPC) proliferation and accelerated neuronal differentiation in schizophrenia. Cortical disorganization, including changes in ventricle form, and disrupted organization of progenitor zones, cortical plate, and cortical layers have been observed in iPSC-derived organoid models of the disorder. Imbalanced differentiation of excitatory and inhibitory neurons has also been detected in patient-derived organoids. The images are schematics based on general findings in the studies cited [22,23,47,104].

Brain connectivity in schizophrenia

Abnormalities in structural and functional brain connectivity have been broadly implicated in schizophrenia pathophysiology. A consistent body of research has reported alterations in both functional and anatomical thalamocortical connectivity in the brains of affected individuals. More specifically, decreased connectivity between thalamus and PFC and increased connectivity between thalamus and sensorimotor cortex have been systematically observed in the brains of patients with schizophrenia [12–14,17]. Notably, thalamocortical miswiring has also been reported in other psychiatric disorders [14,72]. In support of the early developmental origin of these alterations, individuals with familial risk for ASD have been found to display a caudal shift in thalamocortical wiring in infancy [72], whereas toddlers with high risk for schizophrenia reportedly exhibit hypoconnectivity in the thalamo-PFC tract [73]. Longitudinal studies have observed alterations in brain white matter tracts in individuals at high risk for psychosis persisting from childhood to adulthood, with no dramatic changes associated with transition to psychosis [74,75] (Box 2). Thus, the risk for psychosis appears to be linked to developmentally established structural alterations in brain connectivity. In addition to thalamocortical connections, disrupted corticocortical connectivity within the default mode and frontoparietal networks has been observed in patients with schizophrenia [15,16].

Axonal pathways

Dysregulated pathways related to axonal guidance have been among the most common findings in transcriptomic and proteomic iPSC studies of schizophrenia. For instance, alterations in ephrin/Eph signaling and SLIT/ROBO-mediated axonal guidance pathways have been reported in NPCs, neurons, and astrocytes derived from patients with schizophrenia [22,44,48,50,66,85–87]. Despite these indications of altered neuronal wiring in schizophrenia, studies examining axonal growth abnormalities and responsiveness to guidance cues have been rarely performed with iPSC-based models of the disorder. The obvious reason for this is the lack of long-distance growth targets for axonal projections in conventional neuronal monocultures. Instead, decreased neurite length has been frequently reported in patient-derived neurons (Table 1). The growth deficits have been

Box 2. Neuronal connectivity *in vivo* and *in vitro*

In the human brain, the first subcorticocortical axonal pathways emerge by GW8. During the first trimester, subcortical axons cross critical morphogenic sorting points, such as the ventral telencephalon, on their way toward the cortex [4,76]. An important intermediate target for the afferent fibers is the cortical subplate, which functions as a waiting compartment for ingrowing axons [3,38,76]. During GW 13–18, afferent axons from the thalamus, midbrain, and basal forebrain invade the subplate and spread their fibers [3,4,77]. In turn, afferent ingrowth to the cortical plate occurs during the late second trimester and coincides with the migration of subplate neurons to their final cortical position. The final identity and future projection target of cortical long-range projection neurons is also defined in the subplate [3,4,76]. The transcription factors CTIP2 and FEZF2 are required for layer V corticospinal projection neuron specification, whereas the growth of corticothalamic projections from layer VI is orchestrated by SOX5, TBR1, and TLE4 expression. Commissural connections are formed by neurons expressing SATB2 [3,78,79]. The growth of axonal projections is guided by attractant and repellent guidance cues, such as netrins, ephrins, and semaphorins, which neurons express receptors for [80,81].

Cortical long-range projection neuron fate selection has been elegantly demonstrated *in vitro* using human embryonic stem cell (ESC)-derived neurons [3]. The study showed that ESC-derived postmitotic neurons undergo a subplate-stage during which they adopt a corticofucal or corticocortical projection neuron fate. Between days 75 and 120 of differentiation, the neurons were found to co-express the transcription factors NURR1, SATB2, TBR1, and CTIP2 characteristically for subplate neurons. The activity of WNT signaling was identified as a critical factor in neuronal fate selection during this stage. Specifically, WNT inhibition was shown to support the differentiation of SATB2-expressing neurons, whereas WNT activation resulted in differentiation of CTIP2-expressing neurons and TLE4-expressing neurons [3]. In addition to projection neuron differentiation, neuronal circuits have been modeled *in vitro* using compartmentalized culture platforms and brain organoids [82–84]. The development of corticocortical and corticospinal projections has been detected in long-term cultures of cerebral organoids grown on an air–liquid interface [84]. In addition, a differentiation protocol for thalamic organoids was recently introduced and applied to model the formation of thalamocortical and corticothalamic projections in human stem cell-based fused organoids [83].

Table 1. Morphological measures from selected iPSC studies of schizophrenia arranged according to the time points used[a,b]

Measures	Time points																			
	<2 weeks				3 weeks				4–6 weeks						8 weeks		≥3 months			
Refs	[48]	[152]	[130]	[90]		[92]	[145]	[153]	[154]	[89]		[67]	[130]	[90]	[88]	[152]	[44]	[129]	[22]	[88]
Neuronal type	H	C	C	D	D	E	E+M	E	E	E	G	C	C	C	G	C	C	G+ C	C	G
Neurite length	↑	↓	↑	–	↓			–	↑	↓	↓	↓	–	–	↓			–		
Number of primary dendrites				–	↓			–						–	↓		↓			
Branching complexity								–	↑						↓					
Soma size			↑					–					–					–		
Presynaptic puncta density								–		↓	↓	↓	↓	↓			–	↓		
Presynaptic puncta size								–												
Excitatory postsynaptic puncta									–	↓		↓		↓		↓	↓	↓		
Inhibitory postsynaptic puncta											↓						–	↓		
Spine/protrusion density							↓		–										↑	
Mature excitatory synapses						–			↑	↓			↓	↓		–			↓	–
Mature inhibitory synapses											↓								↑	↓

[a]Abbreviations: C, cortical; D, dopaminergic; E, excitatory (NGN2); G, GABAergic; H, hippocampal; M, microglia.
[b]↑ increased in schizophrenia; ↓ decreased in schizophrenia; – no difference in schizophrenia.

associated with reduced expression of adhesion protein-coding genes, including PCDHs, NRXNs, and *NCAM1* [2,88,89]. Given that neurite growth is an energy-demanding process, several studies reporting mitochondrial dysfunction and oxidative stress in patient-derived neurons have also observed deficits in neuritogenesis [64,85,90–92].

Importantly, expression of axonal guidance molecules in the cortex is known to influence targeting of subcortical afferent axons to the appropriate cortical regions. In the mouse brain, thalamic axons bearing the adhesion protein CHL1 and ephrin receptors EPHA3, EPHA4, and EPHA7 are repelled in the ventral telencephalon toward rostral cortical targets by caudally enriched ephrin-A5 and SEMA3A [93,94]. In the somatosensory cortex, ephrin-A5 acts as a repellent toward limbic thalamic fibers that typically innervate the cingulate cortex. Reduced cortical expression of ephrin-A5 causes excess innervation of thalamic fibers to the sensorimotor cortex [95,96]. In several iPSC studies of schizophrenia, altered expression of ephrin-A ligands and receptors was found in cortical neurons and astrocytes from affected individuals [22,44,50,66]. In addition, CHL1 has been identified among the top differentially expressed genes in iPSC-derived neurons and astrocytes from male patients with schizophrenia [24,50]. Altered SLIT/ROBO-mediated axon guidance, which has been detected in patient-derived neurons [44], is also known to distort thalamocortical and corticothalamic axonal targeting as well as corticocortical axonal pathfinding [97]. Altogether, these results suggest that differential expression of axonal guidance molecules by developing brain cells distorts axonal connectivity in schizophrenia.

In addition to axonal pathfinding, the establishment of axonal connections in the human brain continues postnatally through the process of axonal myelination. The myelination process generally starts from caudal regions of the central nervous system and proceeds toward rostral parts of the brain [98,99]. In humans, oligodendrocytes are mostly produced by 5 years of age, after which the myelin thickness continues to increase until adolescence [99]. Given that *in vitro* oligodendrocyte differentiation is time-consuming, only a few studies have investigated oligodendrocytes derived from patients with schizophrenia. These studies reported deficient oligodendrocyte differentiation, morphological maturation, or viability in schizophrenia [100–102]. In one of these studies, iPSC-derived oligodendrocyte precursor cells from patients with schizophrenia were transplanted into the brain of a shiverer mouse [100]. As a result, the patient-specific cells migrated prematurely to the mouse cortex, leading to deficient white matter expansion [100]. Considering the gradual development of myelin, deficient production and misplacement of oligodendrocytes in the brain could lead to uneven myelination and strengthening of axonal pathways in schizophrenia. In general, the current evidence suggests that schizophrenia is associated with persistent developmental alterations in brain connectivity that could be caused by aberrant axonal pathfinding or myelination [74,75]. However, these changes could also arise from remodeling of connections later in adolescence, particularly in somatosensory and prefrontal cortices [103].

Projection neuron specification

In addition to the altered expression of axonal guidance molecules, shifts in cortical long-range projection neuron fate specification have been studied in the context of schizophrenia using neurons carrying DISC1 mutations. In a recent organoid model [104], neuronal postmitotic fate specification failed in long-term cultures of sliced cortical organoids derived from patients with schizophrenia and MD carrying DISC1 mutations. Unlike in control organoids, neurons expressing the layer-specific transcription factors SATB2, TBR1, RORB, and CTIP2 failed to settle into correct cortical lamina in patient-derived organoids after 150 days of differentiation. Second, there was a persistent overlap in the expression of SATB2 and TBR1, and RORB and CTIP2 in the differentiated neurons (Figure 2). Interestingly, corresponding overlap was induced in control organoids by either activating or inhibiting WNT/β-catenin signaling [104]. These results

provide foundational evidence of disrupted establishment of corticocortically and subcortically projecting neuronal populations in schizophrenia. The findings also demonstrate how altered WNT signaling activity not only alters the excitatory–inhibitory balance, but has also broader effects on neuronal fate specification in schizophrenia.

Notably, projection neuron disorganization has been observed in the primary somatosensory cortices of mouse offspring affected by MIA [105]. In the mouse model, cortical disorganization was most prominent in SATB2 and TBR1-expressing projection neurons and was accompanied by loss of PV-expressing interneurons. Stimulating the disorganized neurons that projected to the striatum and temporal cortex induced autism-like repetitive behavioral and social abnormalities in the mouse offspring. These alterations were caused by MIA-induced elevation of maternal IL-17a and subsequent protein translation arrest in the brains of male offspring [105,106]. Conversely to the protein synthesis arrest in the MIA model, a prior iPSC study of schizophrenia detected upregulation of translation initiation factors together with increased total protein synthesis in patient-derived NPCs [107]. In postmortem brain samples, local cortical disorganization has been observed in the PFC and auditory cortices of patients with ASD [10]. All in all, these findings shed light on the importance of cortical area specificity regarding deficits typical for psychiatric disorders and suggest that both known mutations and environmental insults trigger comparable changes in brain development. Interestingly, opposing gene expression alterations appear to result, at least in some cases, in convergent alterations at the circuit-organization level.

Brain functional development in schizophrenia

Electroencephalography and magnetoencephalography studies of schizophrenia have reported alterations in both low-frequency (delta, theta) and high-frequency (gamma) oscillations in the brains of patients with schizophrenia [18,19,108]. Positive symptoms of the disorder have been associated with increased slow wave activity in the temporal lobes [18], whereas decreased gamma oscillatory power at 40 Hz frequency has been characteristic during auditory sensory processing in patients [19,109]. However, the opposite pattern, of decreased slow frequency activity and increased high frequency activity, has also been associated with psychotic symptoms [110]. Since the discovery of NMDA receptor antagonists and their ability to induce psychosis in healthy individuals, a glutamate hypothesis has been one of the prevailing theories of schizophrenia alongside the dopamine theory and other conceptualizations [111]. Both schizophrenia and NMDA receptor antagonist-mediated psychosis are hallmarked by reduced mismatch negativity (MMN), an event-related auditory potential [112,113]. MMN has also been found to characteristically deviate in children at risk of developing schizophrenia, suggesting that the disorder-related alterations in brain function originate early in life [114]. In fact, many of the abovementioned impairments in brain function have been shown to manifest in at-risk individuals before conversion to psychosis and more strongly in those who display conversion to psychosis in the future compared with those who will not [108,109,115] (Box 3).

Excitatory–inhibitory imbalance

The involvement of aberrant glutamate signaling in schizophrenia has been confirmed in multiple iPSC studies. Altered expression of a variety of different glutamate receptor subunits (*GRIN2A*,*B*, *GRIK1-2*, and *GRM1*,*7*) and glutamate transporter genes has been found in iPSC-derived neurons from patients with schizophrenia [2,44,66,68,70]. In addition, numerous iPSC studies have reported altered expression of GABA-synthesizing enzymes and differential expression of GABA receptor subunits in neurons derived from patients with schizophrenia [22,24,44,68,70,129]. These findings imply that deficits in both glutamate and GABA-mediated neurotransmission are involved in schizophrenia pathophysiology. Interestingly, both increased and decreased

Box 3. Brain functional development *in vivo* and *in vitro*

The first detectable oscillations in the brains of preterm human infants are low frequency delta waves (1–4 Hz) that are generated by cortical and thalamic neurons [5,116]. In the cortex, delta waves arise from circuit dynamics involving intrinsically bursting neurons in layer V, and NMDA receptor-mediated excitation by these neurons [116]. During the third trimester, delta waves are coupled with rapid alpha (8–12 Hz), beta (12–30 Hz), and gamma (30–100 Hz) bursts [5]. In the fetal brain, gamma oscillations are thought to be generated through rapid excitation and are replaced by inhibitory $GABA_A$ receptor-mediated adult gamma oscillations after birth [117–119]. Spontaneous gamma waves are generated by neurons in all cortical layers, whereas sensory-driven gamma oscillations at 40 Hz frequency arise mostly from layers III–IV [120,121]. During early adolescence, the delta oscillatory power declines [122]. Simultaneously, sensory cortical gamma oscillatory power at ~40 Hz frequency increases but declines again toward late adolescence [123]. These changes in brain oscillatory patterns coincide with synaptic remodeling and maturation of the inhibitory system in the adolescent brain [8,9].

In human iPSC-based models, the development of neuronal activity from unorganized spike trains into synchronous bursts occurs as early as after 3 weeks of maturation in the presence of astrocytes. Excitatory synaptic inputs involving NMDA and AMPA receptors develop simultaneously [41,124,125]. By contrast, the development of inhibitory activity has been more rarely demonstrated in iPSC-derived neuronal cultures and depends largely on the cell type composition being used and culturing time. Inhibitory activity has been reported in astrocyte-enriched cultures containing a mixture of excitatory and inhibitory neurons [25,126,127]. Impressively, oscillatory patterns typical of late stages of human gestation have been modeled in long-term cultures of iPSC-derived neurons [21,128]. Delta frequency oscillations (2–3 Hz) have been detected in cortical organoids after 4 months of culturing, and the emergence of high-frequency gamma (100–400 Hz) activity has been observed after 6 months of maturation, coinciding with the development of GABAergic neurons [21]. The generation of the oscillatory events has been shown to involve both AMPA and NMDA receptor input, whereas GABAergic input is required to maintain the oscillatory activity [128].

expression of glutamate and GABA signaling-related genes have been reported in affected neurons. Some of this contradiction has been found to stem from sex-specific differences [24].

Based on results from electrophysiological recordings, iPSC-derived neuronal models of schizophrenia have provided evidence of decreased activity in the affected neurons (Table 2). Although only a few iPSC studies have examined the mechanisms of neuronal malfunction in schizophrenia, several studies have found alterations in glutamate- or GABA-mediated responses in the affected neurons in calcium imaging or microelectrode array experiments (Table 2) [24,48,89,129]. Studies investigating synaptic currents through intracellular recordings have further confirmed alterations in excitatory and inhibitory synaptic activity in schizophrenia (Table 2) [25,130,131]. It was recently found that neurons from treatment-responsive patients (typical antipsychotic users) and treatment-resistant patients (clozapine users) exhibited different responses to GABA and glutamate during calcium imaging [24]. Here, neurons from treatment-responsive patients displayed a decreased response to GABA and a normal response to glutamate, whereas the neurons from treatment-resistant patients exhibited an increased response to glutamate and a normal response to GABA [24]. These observations suggest that neuronal dysfunction in schizophrenia can originate from either abnormal excitation or inhibition, depending on the patient's background. Furthermore, preliminary data from a study comparing neuronal activity patterns derived from patients with schizophrenia and the clinical status of the patients found a correlation between altered Na^+ channel dynamics and the positive symptoms of the disorder [25]. In addition, increased inhibitory postsynaptic current frequency was associated with a schizophrenia diagnosis in general [25]. Notably, iPSC-derived models are beginning to shed light on the factors underlying neuronal malfunction in distinct phenotypes of schizophrenia.

NMDA receptor hypofunction

NMDA receptor antagonist-induced psychosis has been used as a model of schizophrenia in which aberrant neuronal interactions in cortical disinhibitory circuits have been linked to the brain malfunction [132,133]. More specifically, inhibitory neurons are thought to receive attenuated input from excitatory neurons and thereby fail to effectively inhibit their target excitatory neurons, leading to neuronal hyperactivity. The fact that NMDA receptor antagonists can induce

Table 2. Electrophysiological measures from selected iPSC studies of schizophrenia arranged according to the time points used[a,b]

Measures	Time points																	
	NPC	3–4 weeks		4–6 weeks							8 weeks – 3 months						≥4 months		
Refs	[48]	[153]	[155]	[131]	[89]		[130]	[92]	[82]	[67]	[152]	[25]	[24]		[44]	[129]	[22]	[88]	[68]
Neuronal type	H	E	E/C	H	E	G	C	E	H	C	C	C	C[c]	C[d]	C	G+C	C	G	O
EPSC frequency		m ↓		s ↓			↓					s –					m –	s –	
EPSC amplitude		e ↓		s ↓			↓					s –					m –	s –	
IPSC frequency												s ↑					m –		
IPSC amplitude												s –					m –	e –	
Resting membrane potential		–						–				–						↑	
Input/membrane resistance		–						–	↓			↑						–	
Membrane capacitance		–										–						–	
K^+ current density		–							–			–						–	
Na^+ current density		–							–			–						–	
Na^+ current threshold (peak 2)												↓							
Neuron excitability		–							↓	↓		–						–	
Action potential threshold		–										–						–	
Calcium transient amplitude						–					–				–				
Calcium transient rate						–					↓				–				
Number of active neurons				↓						↓									
Spontaneous firing rate			↑		–				↓	↓						↓			–
Network bursting rate									↓										
Network synchrony									↓						–				
Glutamate response	↓												↑	–					
GABA response					↑								–	↓		↓			
Response to NMDAR blocking					–														
Response to AMPAR blocking					↑														

[a]Abbreviations: AMPAR, AMPA receptor; C, cortical; D, dopaminergic; E, excitatory (NGN2); EPSC, excitatory postsynaptic current; e, evoked; G, GABAergic; H, hippocampal; IPSC, inhibitory postsynaptic current; m, miniature; NMDAR, NMDA receptor; s, spontaneous.
[b]↑ increased in schizophrenia; ↓ decreased in schizophrenia; – no difference in schizophrenia.
[c]Neurons from treatment-resistant patients.
[d]Neurons from treatment-responsive patients.

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overactivation of cortical neurons in rats supports this theory and suggests that inhibitory neurons are more likely to be inhibited by these compounds than are excitatory neurons [132]. In line with this notion, it was recently demonstrated that the NMDA receptor antagonist ketamine can drive disinhibition by blocking NMDA receptors in somatostatin (SST)-expressing interneurons that inhibit dendrites of superficial layer excitatory neurons in the mouse PFC [133]. Furthermore, *GluN2B* knockdown in the SST-expressing neurons largely mimicked ketamine-induced disinhibition in the mouse brain [133].

SST-expressing cortical interneurons also receive inhibitory input from vasoactive intestinal peptide (VIP)-expressing interneurons that relay signals from long-range corticocortical projections during sensory processing [134,135]. Pituitary adenylate cyclase-activating polypeptide (PACAP) is a neuropeptide that closely resembles and shares receptors with VIP [136]. It was recently shown with two separate data sets that *ADCYAP1*, which encodes the PACAP protein, was involved in the majority of top causal pathways that were dysregulated in iPSC-derived neurons from patients with schizophrenia [70]. PACAP is known to enhance NMDA receptor-mediated activity by activating cAMP/PKA signaling, which leads to RACK1 release from GRIN2B subunits of NMDA receptors [137]. A high-affinity PACAP receptor, PAC_1, is expressed by all SST- and PV-expressing interneurons, and by most of the layer II/III and V glutamatergic neurons in mouse cortex [138]. Hence, PACAP receptors are robustly expressed in neuronal subtypes involved in disinhibitory control. All in all, schizophrenia-related PACAP deficiency could result in reduced NMDA receptor-mediated activity, especially in SST-expressing interneurons that are highly enriched with VIP/PACAP receptors and GRIN2B subunits.

In addition to PACAP, dysregulation of cAMP-synthesizing adenylyl cyclase enzymes (ADCY1-9) and cAMP-degrading phosphodiesterase (PDE) enzymes has been observed in neurons derived from patients with schizophrenia [22,44]. Recently, the PDE4 inhibitor rolipram was used to rescue synaptic deficits and reduced excitatory synaptic activity in iPSC-derived neurons carrying DISC1 mutations, as well as social and cognitive deficits in mice with the same mutation [139]. PDE4 inhibitors have also been shown to improve deficits in MMN and working memory-related theta activity alterations clinically [140]. Interestingly, preliminary data linked schizophrenia-associated *SETD1A* mutation to hyperactivity in the cAMP/PKA pathway in iPSC-derived excitatory neurons [141]. As done in this study, it is critical to consider cell type specificity when investigating neurobiological phenomena arising from excitatory–inhibitory neuronal interactions. In addition to glutamate signaling, PDE4 is known to modulate dopaminergic neurotransmission through the dopamine D1 receptor/PKA/DARPP-32 pathway [142]. Hence, cAMP/PKA signaling could function as a link between the glutamate and dopamine hypotheses of schizophrenia.

Synaptic remodeling

In the developing human PFC, synaptic density increases significantly until childhood and peaks before 10 years of age. During adolescence, the number of synapses decreases gradually and continues to decrease until the third decade of life [9]. Microglial cells, the resident macrophages of the brain, have an important role in pruning excess synaptic connections [143]. Several lines of evidence support the hypothesis that aberrant synaptic remodeling could be linked to schizophrenia. First, the typical age at onset for schizophrenia is from the early 20s to 30s following the peak period of synaptic pruning [6,7]. Second, postmortem studies of schizophrenia have reported decreased density of postsynaptic elements in the superficial PFC layers [31].

iPSC studies of schizophrenia have consistently reported decreased numbers of pre- and postsynaptic elements in cultures of patient-derived neurons. Among the most consistent observations in these studies has been the reduced density of excitatory postsynaptic elements in the

affected neurons (Table 1), concordantly with genetic studies, which have identified genes encoding postsynaptic proteins as schizophrenia risk genes [144]. These results suggests that synapse deficiency in schizophrenia originates during early brain development and is primarily associated with the excitatory postsynaptic compartment. However, in some studies that have reported synaptic deficits, reduced expression of adhesion genes, including PCDHs and *NLGN2*, have been found specifically in inhibitory iPSC-derived neurons [88,129].

In addition to altered synaptogenesis, impaired synaptic pruning has been demonstrated *in vitro* using iPSC-derived neurons and monocyte-derived microglia-like cells from patients with schizophrenia [145]. The study uncovered that a schizophrenia risk variant in the *C4* locus of the MHC region is associated with increased synaptic deposition of complement protein C3, which functions as a tag for synaptic elimination [145]. This and other studies demonstrated how cell–cell interactions can have a central role in the neuropathology of schizophrenia. For instance, interactions between neuronal and glial cells are required for the circuit-remodeling effects of the complement cascade [146]. All in all, incorporation of glial cells into *in vitro* models of psychiatric disorders could improve their relevance for disease modeling.

The expression of mitochondrial genes has been shown to follow closely the course of spine density changes across postnatal brain development [147]. In addition, brain glucose uptake increases during early childhood and decreases toward late childhood, in a trajectory reminiscent of changes in synaptic density in the PFC [9,148]. In addition to deficits in synaptic development as discussed earlier, various iPSC studies have linked mitochondrial dysfunction to schizophrenia [64,85,90–92,149,150]. Such changes have been consistently highlighted in patient-derived neuronal models of 22q11.2 deletion syndrome [92,149,150]. Not surprisingly, the deleted region contains multiple mitochondrial genes, including *PRODH*, *MRPL40*, *TANGO2*, *ZDHHC8*, *SLC25A1*, and *TXNRD2* [151]. A recent iPSC study of 22q11.2 deletion syndrome investigated neurons derived from deletion carriers with and without schizophrenia [150]. Here, the neurons derived from patients with schizophrenia displayed reduced ATP levels and electron transport chain complex I and IV activity compared with neurons from deletion carriers without schizophrenia. Instead, the neurons from deletion carriers without schizophrenia expressed elevated expression levels of electron transport chain genes compared with neurons from healthy controls. In other words, neurons from deletion carriers without schizophrenia appear to have protective features that may compensate for the mitochondrial dysfunction linked to the deletion [150]. Altogether, these findings support the notion of mitochondrial dysfunction as a major risk factor for schizophrenia. It would be interesting in future work to study schizophrenia-linked mitochondrial dysfunction in the context of synaptic maturation, given the high energy demands involved in synaptic remodeling.

Concluding remarks

Human iPSC studies of schizophrenia have found evidence of cortical maldevelopment starting from neurogenesis. An increasing number of studies have linked altered WNT signaling activity to abnormal NPC proliferation, imbalanced differentiation of excitatory and inhibitory cortical neurons, and failure to specify layer-specific projection neuron identities [22,46–48,104]. Postmortem and animal models of psychiatric disorders have detected comparable abnormalities in the PFC and somatosensory cortices of affected individuals [10,105]. Furthermore, a large body of recent imaging studies has reported thalamic miswiring to these cortical regions in psychiatric disorders [12–14,17]. Together with postmortem and animal studies, iPSC studies have also linked prenatal immune activation to impaired inhibitory system development and cortical disorganization typical of psychiatric disorders [61,63,64,104]. All in all, iPSC-based models have added a layer of understanding to the developmental origins of schizophrenia by

Outstanding questions

Does the imbalance between excitatory and inhibitory systems in schizophrenia arise during neurogenesis? A preference toward excitatory or inhibitory neuronal differentiation has been detected in brain organoids modeling the disorder. However, a more reliable representation of neuronal subtype development and migration to the cortex could be accomplished with fused cortical and ventrally patterned organoids.

Are the alterations in brain connectivity in schizophrenia established already prenatally? Miswiring of connections from the thalamus to prefrontal and sensory cortical regions is a largely acknowledged but understudied feature of the disorder. Current evidence suggests that these alterations have an early developmental origin that could be studied with patient-specific models of axonal pathfinding and myelination.

What is the contribution of environmental factors to the development of schizophrenia? Animal and iPSC-based models have linked, for instance, prenatal immune insults to altered cortical development and transcriptomic changes typical to psychiatric disorders. Although both genetic liability and environmental insults are known risk factors of schizophrenia, models investigating the cooperative effects of the two are still largely missing.

What are the neurobiological mechanisms underlying neuronal malfunction in schizophrenia? Studies using patient-derived neurons have linked altered excitatory and inhibitory neurotransmission to distinct disease phenotypes. In the future, the cell type specificity of these alterations should be investigated, and the background of the patients should be considered when interpreting the results.

What is the role of glial cells in the development of schizophrenia? The importance of astrocytes and microglia in synaptic maturation and pruning has been evidenced with iPSC-based models. These processes are affected in schizophrenia and should be studied using co-cultures of patient-derived neurons and glial cells.

providing insights into signaling pathways disrupted in the disorder and their potential implications for neuronal circuit miswiring. In the future, methodological advances in iPSC models could help address additional questions regarding neural circuit development and disruption in schizophrenia. For instance, fused cortical and ventrally specified organoids, instead of whole-brain organoids that lack distinct brain regions, could be used to examine the development of excitatory and inhibitory neurons, and their migration to the cortex (see Outstanding questions). In addition, circuit-scale models comprising cortical and subcortical neurons could be used to test the causality between altered cortical neurogenesis and miswiring between brain regions in schizophrenia. Patient-specific iPSC-derived brain cells also provide an excellent platform for studying the interplay between the genetic risks for schizophrenia and environmental insults during prenatal development.

Functional and transcriptomic characterization of patient-derived neurons have revealed an imbalance in the development of excitatory and inhibitory systems in schizophrenia [2,22,24,25, 44,66,68,70,129]. The studies also provided not only evidence of excitatory post-synaptic deficits, concordantly with postmortem and genetic findings [31,144], but also emerging evidence of altered inhibitory synaptogenesis [88,129]. The possible contribution of altered cAMP signaling to neuronal dysfunction has been acknowledged clinically and studied recently using neuronal *in vitro* models of schizophrenia [139,141].

Although functional characterization of patient-derived neurons has been performed in an increasing number of studies, relatively few of these studies have involved mechanistic investigation of the role of different neurotransmitter receptors, ion channels, and cell types in neuronal functional alterations. To reveal whether an imbalance between glutamate and GABA-mediated neurotransmission or altered ion channel function contribute to the early neuronal malfunction in patient-derived neurons, neuronal functional development should be characterized more comprehensively, cell type specifically, and considering the background of the patients. To investigate whether deficient cAMP signaling could underlie NMDA receptor hypofunction and neuronal disinhibition in schizophrenia, circuit-scale models comprising excitatory and inhibitory neuronal subtypes would be needed. Given that glial cells function as pacers of synaptic maturation (Figure 1), they may also critically participate in neuronal malfunction in schizophrenia, as early evidence from iPSC studies suggests [50,100,145]. Fuller integration of glial cell types with iPSC-derived neurons could open new avenues for investigating the neurodevelopmental origins of the disorder.

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Authors' contributions

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Declaration of interests

The authors declare no competing interests.

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