Generating a Functional Human Cortex *In Vitro* From Induced Pluripotent Stem Cells

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Introduction

Progress in understanding the development of the human nervous system and elucidating the mechanisms of mental disorders has been greatly limited by restricted access to functional human brain tissue. In recent years, a paradigm shift has been achieved with the introduction of cellular reprogramming—a process in which terminally differentiated somatic cells can be converted into pluripotent stem cells, named human induced pluripotent stem cells (hiPSCs) (Takahashi and Yamanaka, 2006; Takahashi et al., 2007). These hiPSCs can be generated from any individual and can be directed to differentiate *in vitro* into derivatives representing all germ layers, including neural cells. Numerous methods have been developed for the directed differentiation of human neurons from pluripotent stem cells (Paşca et al., 2014; Tabar and Studer, 2014). These approaches use defined conditions to mimic the specific *in vivo* developmental events that give rise to the diverse subtypes of neurons in the brain (Fig. 1). The neural cells derived *in vitro* can be used to study not just normal neuronal function, but to understand how different cell types are affected in disorders of the brain and to develop potential cell-replacement strategies.

Generation of Excitatory Cortical Neurons From hiPSCs

Cortical excitatory neurons are born in the dorsal forebrain, arising from actively dividing neural progenitor cells called radial glia. Within the ventricular zone (VZ), radial glia integrate cell-intrinsic and cell-extrinsic signals, undergoing characteristic modes of cell division during neurogenesis (Mione et al., 1997; Noctor et al., 2004; Farkas and Huttner, 2008). Deeper-layer neurons are generated first, followed by the birth of superficial-layer neurons (Leone et al., 2008). In humans and nonhuman primates, studies have described an enlarged proliferative zone called the outer subventricular zone (oSVZ). This structure is home to outer radial glia (oRG) progenitor cells, which may underlie some aspects of cortical expansion in the primate lineage (Fietz et al., 2010; Hansen et al., 2010; LaMonica et al., 2012; Betizeau et al., 2013). The combinatorial expression of lineage-specific transcription factors has been used to delineate the laminar identity of individual classes of excitatory cortical projection neurons (Hevner et al., 2001; Arlotta et al., 2005; Chen et al., 2005; Molyneaux et al., 2005; Alcamo et al., 2008; Britanova et al., 2008; Lai et al., 2008; Leone et al., 2008; Molyneaux et al., 2009). For example, expression of the transcription factor CTIP2 (also known as BCL11B) is required for the specification of subcortically projecting neurons, whereas cells expressing SATB2 are thought to project callosally to the contralateral hemisphere (Alcamo et al., 2008; Britanova et al., 2008).

Several approaches have been developed for differentiating human pluripotent stem cells (hiPSCs or human embryonic stem cells [hESCs]) into cortical excitatory neurons (Figs. 1*b, c*). Forebrain identity is likely a default state for neuronal specification of pluripotent human stem cells, and existing protocols yield neurons with a rostral identity without exogenous morphogen application (in hESC/hiPSC cultures, inhibitors of SHH [sonic hedgehog] signaling such as cyclopamine are not required). Some of these methods achieve neural induction in high-density monolayer cultures (Shi et al., 2012; Chambers et al., 2009) or by embedding clusters of hiPSCs in gelatinous protein mixtures (e.g., Matrigel, BD Biosciences, Erembodegem, Belgium) and later culturing them in a spinning bioreactor (Lancaster et al., 2013). Other approaches use embryoid bodies derived from hiPSCs that are either plated on coated surfaces to generate neural progenitors organized in rosettes (Li et al., 2009; Marchetto et al., 2010; Brennand et al., 2011; Paşca et al., 2011) or maintained in suspension initially in serum-free conditions and later in serum and Matrigel (for example, SFEBq: serum-free floating culture of embryoid body–like aggregates with quick reaggregation, first described in rodent ESCs by Eiraku et al., 2008) (Mariani et al., 2012; Kadoshima et al., 2013).

We recently reported a simple method for generating pyramidal neurons from hiPSCs in a functional three-dimensional (3D) cerebral cortex–like structure (Paşca et al., 2015). These neural structures, which we named human cortical spheroids (hCSs), were generated from intact hiPSC colonies that were cultured and minimally patterned in exclusively nonadherent conditions and in the absence of extracellular scaffolding. The hCS method generated only excitatory neurons of the dorsal telencephalon. Moreover, the internal cytoarchitecture was reminiscent of a laminated neocortex and grew to include equal proportions of projecting neurons expressing deep-layer and superficial-layer cortical markers. Transcriptional analysis and comparison with the developing human brain revealed that hCSs after 2.5 months resembled the midfetal prenatal brain at 19–24 postconception weeks (PCW). Cortical neurons were accompanied by a network of nonreactive astrocytes and were synaptically connected. Importantly, hCSs were amenable to

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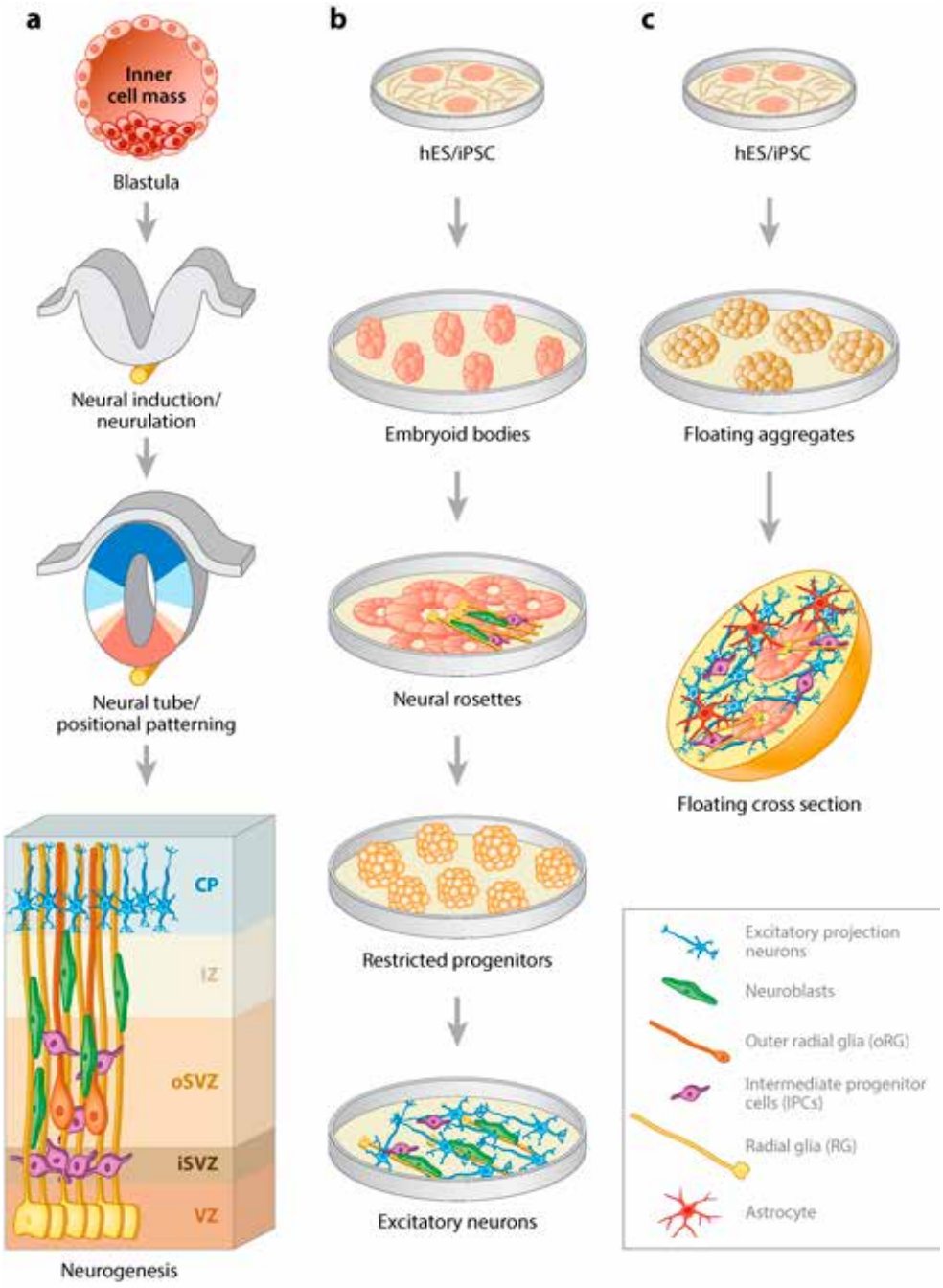

Figure 1. Approaches for the generation of excitatory cortical projection neurons. ***a***, The developmental events underlying the generation of cortical excitatory neurons *in vivo* are recapitulated *in vitro* using (***b***) adherent cultures or (***c***) suspended aggregate methods. Reprinted with permission from Paşca et al., 2014, their Fig. 2.

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acute-slice physiology, making it possible to record and electrically stimulate neurons while preserving a relatively intact network. Lastly, this method is scalable and reproducible between hiPSC lines, across and within differentiations. hCSs have the potential to reveal cellular phenotypes associated with neuropsychiatric disorders, identify biomarkers for early diagnosis and clinical stratification, and provide a platform for drug and teratologic agent screenings *in vitro*.

To generate suspended cellular aggregates of pluripotent cells, we used cultures of hiPSCs grown on feeders. Rather than using single-cell suspensions, we enzymatically detached intact hiPSC colonies from inactivated feeders (Fig. 2*a*). Suspended colonies were subsequently transferred into low-attachment plates in a KnockOut Serum–based medium (ThermoFisher Scientific, Waltham, MA) without fibroblast growth factor 2 (FGF2). Within a few hours, the floating hiPSC colonies folded into spherical structures. To achieve rapid and efficient neural induction, both the bone morphogenetic protein (BMP) and transforming growth factor beta (TGF-β) signaling pathways were inhibited with small molecules: dorsomorphin (also known as compound C) and SB-431542. On day 6 in suspension, the floating spheroids were moved to serum-free Neurobasal Medium with Gibco B-27 supplement (ThermoFisher Scientific) containing FGF2 and epidermal growth factor (EGF). By day 18, more than 85% of cells expressed PAX6, and more than 80% of these progenitors expressed FOXG1. To promote differentiation of neural progenitors into neurons, starting at day 25, FGF2 and EGF were replaced with brain-derived neurotrophic factor (BDNF) and neurotrophic factor 3 (NT3). From day 43 onward, only neural medium without growth factors was used for medium changes every 4 days. After approximately 7 weeks of differentiation

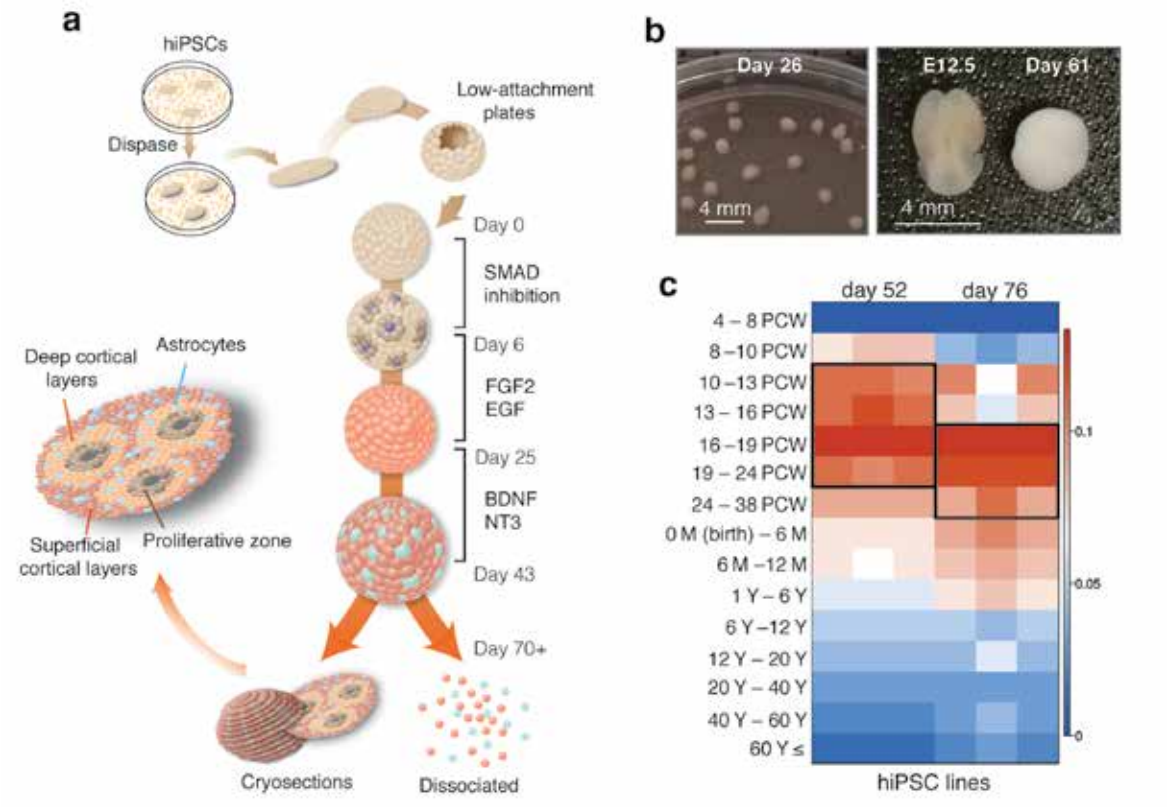

Figure 2. Generation and characterization of hCSs from hiPSCs. ***a***, Scheme illustrating the main stages of the method for generating hCSs from hiPSCs. Floating hCSs can be either dissociated for flow cytometry or monolayer culture or fixed and sectioned for immunofluorescence experiments. ***b***, Morphology and size of hCSs at days 26 and 61 *in vitro*. For size comparison at day 61, a dissected E12.5 mouse brain is shown. Scale bars, 4 mm. ***c***, Transcriptional analyses and mapping onto the developing and adult human brain of hCSs at days 52 and 76 using the machine-learning algorithm CoNTExT (n = 3 hiPSC lines per time point from 3 subjects). Modified with permission from Paşca et al., 2015, their Fig. 1.

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in vitro, 78.8% ± 2.5% of the cells expressed the neuronal marker β3-tubulin (TUBB3), and 36.2% ± 3.6% of neurons expressed the mature neuronal marker NeuN (neuronal nuclear antigen), which is present in the human forebrain only after 20 weeks of gestation (Sarnat et al., 1998). The hCSs grew in size up to 4 mm in diameter by 2.5 months (4.2 ± 0.3 mm) (Fig. 2*b*).

We used transcriptional profiling to assess developmental maturity and observed a strong overlap between hCSs and cortical developmental stages up to late midfetal periods (19–24 PCW) (Fig. 2*c*). This was in contrast to monolayer methods as well as other 3D approaches for neural differentiation of hiPSCs (Mariani et al., 2012; Stein, 2014) that yield neurons mapping onto earlier fetal stages. When we looked for genes whose expression was changing in the same direction in hCSs and human fetal cortex between stages 1 and 2 (4–10 PCW) and stage 6 (19–24 PCW), but not in hiPSC-derived neural cultures differentiated in monolayer, we found that upregulated genes were enriched for synaptic transmission genes, whereas the downregulated genes were enriched for cell-cycle and cell-division genes.

When we examined the cytoarchitecture of the hCSs (day 52) in cryosections, we observed proliferative zones containing PAX6-expressing progenitors (Fig. 3*a*). Similarly to what occurs during *in vivo* cortical development, VZ-like structures inside hCSs were organized around a lumen delimited by N-cadherin (Ncad)–expressing cells. Furthermore, the VZ-like zone was surrounded by an intermediate zone (IZ) rich in $TBR2^+$ (T-box brain protein 2) cells resembling the SVZ (Fig. 2*b*). PAX6-expressing cells in the VZ-like zone also contained $GFAP^+$ extensions directed orthogonally to the luminal surface, resembling radial glia (Fig. 3*c*). When plated in monolayer, these cells had either bipolar or monopolar morphologies (Figs. 3*d*, *e*). Both $PAX6^+$ and $TBR2^+$ neural progenitors were actively proliferating, as assessed by the expression of the radial glia–specific mitotic marker phospho-vimentin (pVIM) and the G2/M phase marker phosphohistone-3 (PH3) (Figs. 3*f*, *g*). In a pattern similar to *in vivo* cortical development, most of these mitoses were localized close to the luminal side of the proliferative zone rather than being dispersed. Live imaging of radial glia fluorescently labeled with a cell-specific reporter (lentivirus expressing EGFP under the human GFAP promoter [Lenti-GFAP::EGFP]) revealed a characteristic division mode reminiscent of interkinetic nuclear migration (Fig. 3*h*).

On the surface of the hCSs, we observed a layer of horizontal cells expressing reelin (RELN), suggestive of a marginal zone (Fig. 3*i*). The TBR1 protein, localized in the subplate (SP) and cortical plate (CP), and later in layers V–VI (Saito et al., 2010), reached a peak of expression at day 76 (equivalent of SP and inner CP) (Fig. 3*j*). CTIP2, a transcription factor involved in specifying subcortical projection neurons (Alcamo et al., 2008; Britanova et al., 2008), was highly expressed early in hCSs, and decreased over time after the *in vitro* stage equivalent to the early midfetal period. In contrast, superficial-layer cortical markers increased up to sevenfold from day 52 to day 137 *in vitro*. The transcription factor BRN2 (POU3F2), which is expressed in late cortical progenitors and migrating neurons, reached a peak of expression earlier than SATB2, which defines corticocortical projecting neurons. The relative proportion of superficial-layer neurons was also confirmed by the expression of the homeodomain family proteins CUX1 and CUX2, whose expression is localized mostly to layers II–IV. We found that the generation of these neurons is highly reproducible among hiPSC lines, and within and across differentiations of the same hiPSC line.

We noticed that layer-specific cortical neurons in hCSs were organized concentrically around a VZ-like zone (Figs. 3*k*, *l*). Deep-layer neurons expressing TBR1 and CTIP2 moved immediately outside the proliferative zone, whereas at *in vitro* day 137, superficial-layer cortical neurons expressing SATB2 and BRN2 had migrated farther away, forming the outside layer of the hCSs. Flow cytometry experiments indicated that the mutually exclusive CTIP2 and SATB2 proteins, which regulate alternate corticofugal and corticocortical cellular identity programs, were coexpressed by fewer than 3% of the cells. Labeling with EdU (5-ethynyl-2'-deoxyuridine) showed that most of the superficial-layer neurons are formed after 8 weeks of differentiation *in vitro*. This process continues for at least another 7 weeks, until the proportion of superficial-layer and deep-layer neurons is approximately equal (Fig. 3*j*).

Generation of Astrocytes in 3D Cultures

Both neurons and astrocytes share a common neuroepithelial origin and are born throughout embryogenesis in a temporally defined manner (Bayer and Altman, 1991; Shen et al., 2006). We hypothesized that because of the longer exposure to FGF2 and EGF, progenitors in the proliferative zones of hCSs would ultimately undergo a neurogenesis-to-

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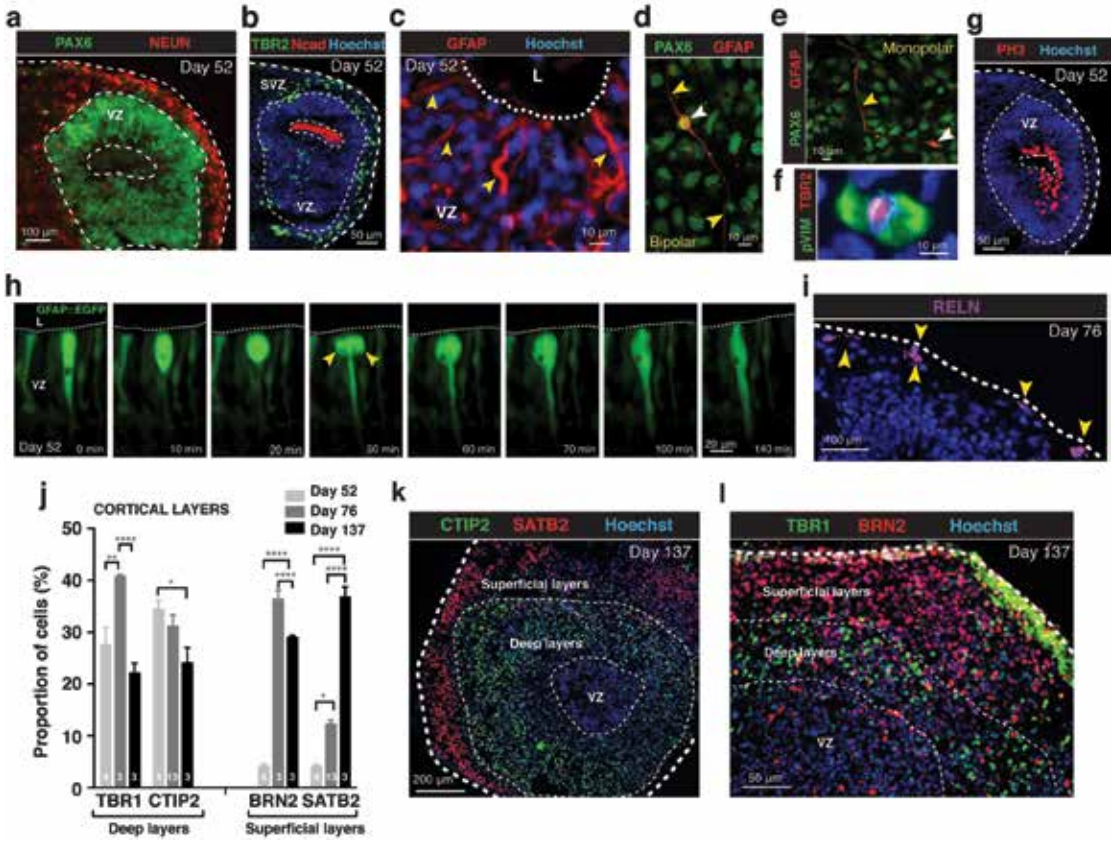

Figure 3. Corticogenesis in the hCS. ***a***, Cryosection of an hCS at day 52 stained for PAX6 (progenitors) and NEUN (neurons), demonstrating the presence of a VZ-like region organized around a lumen. ***b***, Intermediate progenitor cells (TBR2$^+$) are present in a SVZ-like region beyond the VZ; Ncad stains the luminal side of the progenitors. ***c–f***, Radial glial cells expressing combinations of GFAP, PAX6, or TBR2 and pVIM are present in proliferative zones, extend processes perpendicular to the lumen (L) and, when plated in monolayer, have either one or two processes. White arrowheads indicate the cell body and yellow arrowheads the processes. ***g***, Mitoses (PH3$^+$) are spatially restricted to the luminal side of the proliferative zones. ***h***, Live imaging showing interkinetic nuclear migration (Lenti-GFAP::EGFP). ***i***, RELN$^+$ neurons are positioned horizontally on the surface of hCSs. ***j***, Quantification in cryosections of the proportion of cells expressing layer-specific cortical markers at three time points of differentiation (mean ± SEM; n = 3–13 hCSs [numbers listed within each bar] from 4 hiPSC lines derived from 4 individuals; 2-way ANOVA, $F_{2,48}$ = 32.96, $p < 0.0001$ for time point [day 52 vs day 76 vs day 137]; Tukey's multiple-comparison tests: *$p < 0.05$, **$p < 0.01$, ****$p < 0.0001$). ***k***, ***l***, Cryosections of hCSs at 137 d stained for the indicated markers, showing organization of layer-specific neurons. Scale bars: ***a***, ***i***, 100 µm; ***b***, ***g***, ***l***, 50 µm; ***c–f***, 10 µm; ***h***, 20 µm; ***k***, 200 µm. Modified with permission from Paşca et al., 2015, their Fig. 2.

gliogenesis switch. After 7 weeks of differentiation *in vitro*, we noticed astrocytes with thin GFAP$^+$ processes intermingled with neurons cells in the hCS parenchyma (Fig. 4*a*). As expected, we observed few GFAP$^+$ cells during the first 35 days of differentiation (Figs. 4*b*, *c*), but this proportion increased to approximately 8% by day 76 and almost 20% after 180 days (Fig. 4b). We also closely examined the morphology of GFAP$^+$ cells after dissociation. When maintained in monolayer in defined serum-free culture conditions (Foo et al., 2011), astrocytes extended abundant thin projections (Fig. 4*d*). To investigate whether these cells could respond to reactive cues *in vitro*, we added serum, which is a potent activator of reactive astrogliosis, to the culture medium. Within several days, the cells adopted a reactive phenotype with polygonal morphologies and upregulated expression of genes associated with *in vivo* astrogliosis, including GFAP, VIM, and LCN2 (lipocalin 2). Finally, using electron microscopy, we confirmed that the thin GFAP$^+$ processes dispersed throughout the hCSs contained numerous glycogen granules, which are localized predominantly in astrocytes in the mammalian brain (Brown and Ransom, 2007).

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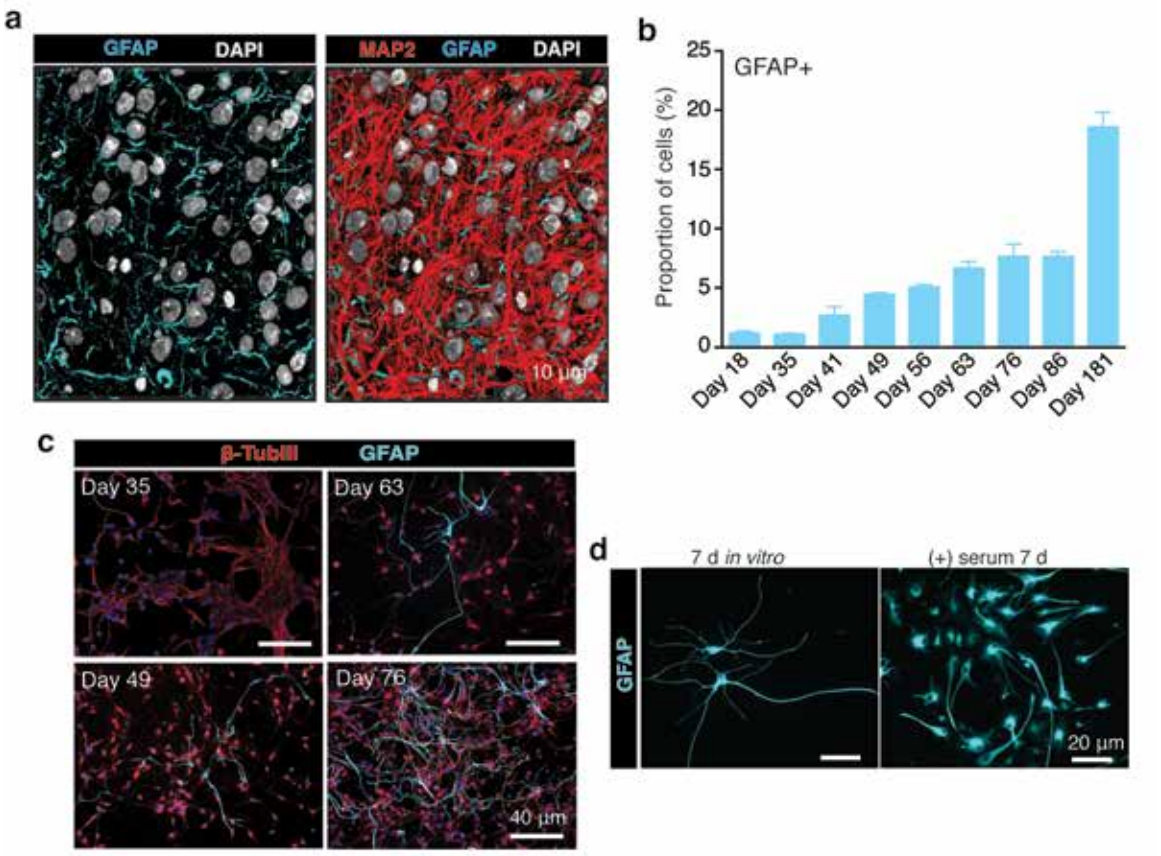

Figure 4. Astrogenesis in cortical hCSs. ***a***, Volume rendering by array tomography of the interior of an hCS (74 × 88 × 2.45 µm) revealing the commingling of MAP2 (red) staining of neuronal dendrites and GFAP (cyan) staining of glial processes. DAPI staining for nuclei is rendered in white. ***b–c***, Developmental time course for the generation of GFAP$^+$ cells. Quantification performed in dissociated hCSs (mean ± SEM; n = 3 for all time points except day 63, when n = 4; ANOVA $F_{8,19}$ = 66.75, p < 0.0001). ***d***, Astrocyte morphology after the indicated periods of *in vitro* culture in monolayer in defined, serum-free medium and after 1-week exposure to serum. Scale bars: ***a***, 10 µm; c, 40 µm; ***d***, 20 µm. Modified with permission from Paşca et al., 2015, their Fig. 3.

Synaptogenesis and Functional Assessment of 3D Cortical Spheroids

We also tested the ability of the cells in hCSs to differentiate into electrically active mature neurons. We observed that neurons displayed abundant spontaneous calcium spikes and found that all neurons recorded by patch–clamping produced a transient inward current after depolarization beyond –30 mV, which was blocked by tetrodotoxin (TTX). This inward voltage-gated Na^+ current was followed by activation of a more sustained K^+ current. Importantly, depolarizing current injection revealed that all the recorded neurons reliably produced action potentials (Fig. 5*a*).

Considering the functional maturity of the cortical neurons and the presence of astrocytes, we investigated synaptogenesis within 180-day-old hCSs. To identify individual synapses with a high degree of confidence, we used array tomography to visualize individual synapses (Micheva and Smith, 2007) within the dense neuropil of the hCSs and antibodies against multiple presynaptic and postsynaptic proteins. We found that the presynaptic protein synapsin-1 (SYN-1) and postsynaptic protein PSD-95 were expressed throughout the interior of the hCSs in both large and small puncta (Fig. 5*b*). Larger SYN-1 puncta were often found adjacent to PSD-95 puncta, indicating the presence of a synapse. To examine these larger puncta in further detail, we constructed synaptograms consisting of a series of high-resolution sections through a single synapse where we could probe for potential colocalization of at least three independent synaptic markers. In many cases, we observed colocalization of the presynaptic proteins SYN-1 and the glutamate transporter VGLUT-1 in close apposition to the postsynaptic protein PSD-95,

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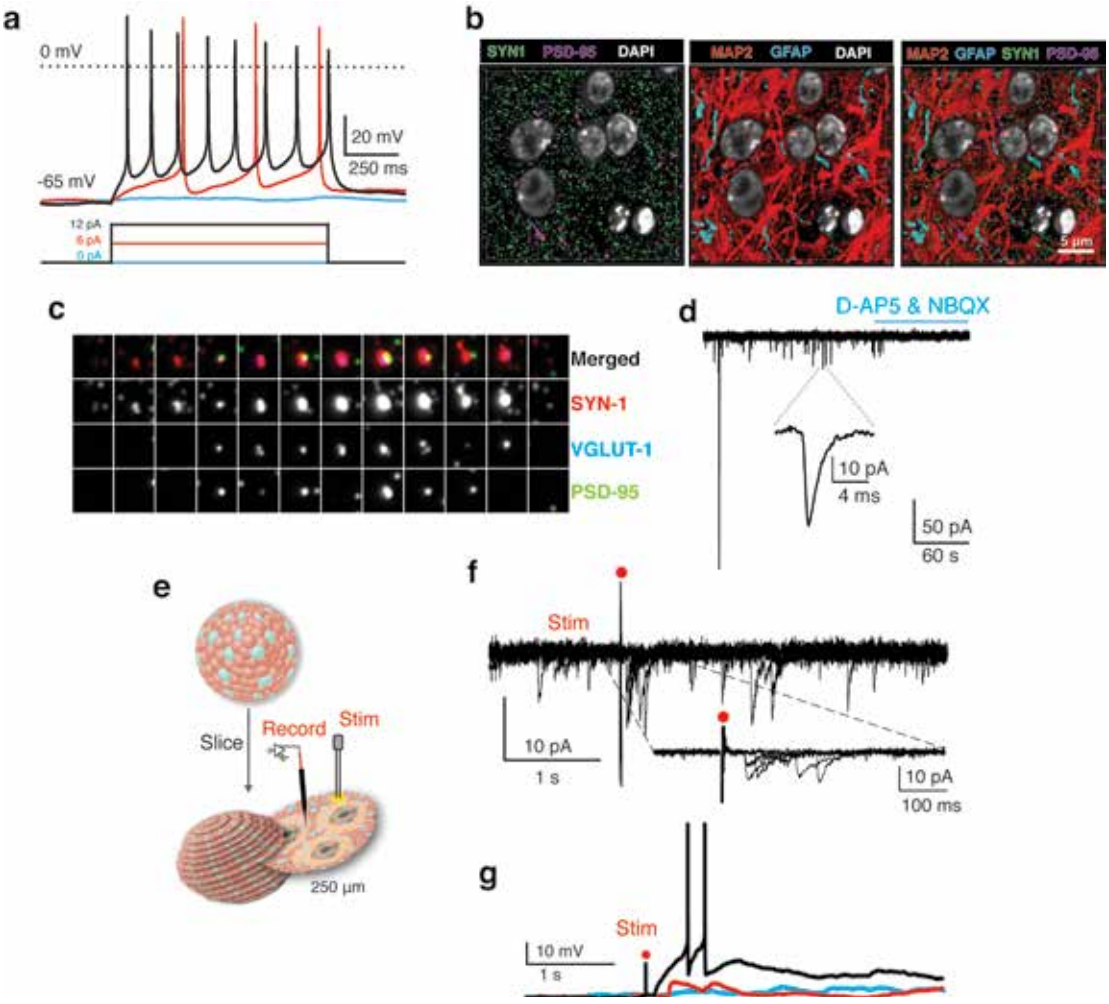

Figure 5. Synaptogenesis and functional characterization of cortical neurons from hCSs. ***a***, Representative trace of a whole-cell current-clamp recording in an acute hCS slice preparation. Current injections (6 or 12 pA steps from –65 mV) produce sustained action potential generation. ***b***, The distribution of structural (MAP2, GFAP) and synaptic proteins (SYN-1, PSD-95) inside hCSs visualized with array tomography (volume: 29 × 29 × 2.45 µm). Scale bar: 5 µm. ***c***, Synaptogram (70 nm sections) revealing a synapse inside a hCS. Twelve consecutive sections are represented in each row, and different antibody stains for the same section are represented in each column. ***d***, Representative traces of spontaneous EPSCs recorded at –70 mV in neurons derived in hCSs and cultured in monolayer for 2 weeks, testing the effect of 25 µM NBQX and 50 µM D-AP5. ***e***, Schematic illustration of slicing of hCSs, electrophysiological recordings (Record), and stimulation (Stim). ***f***, Voltage-clamp recordings showing EPSCs after electrical stimulation in an acute hCS slice preparation. Composite of seven overlaid sweeps from a neuron. Inset shows stimulus-evoked EPSCs at higher temporal resolution. The electrical stimulation artifact is designated by a red dot. ***g***, Current-clamp recordings of action potentials (black trace), EPSPs (red trace), and failures (blue trace) evoked by electrical stimulation (red dot) of hCS slices. Calibrations: ***f***, 10 pA, 1 s (left), 10 pA, 100 ms (right); ***g***, 10 mV, 1 s. Modified with permission from Paşca et al., 2015, their Fig. 5.

indicating the presence of a glutamatergic synapse (Fig. 5*c*). In some cases, the NMDA receptor subunit NR2B was also colocalized with PSD-95.

We found that the majority (88.8%) of neurons exhibited spontaneous synaptic activity. This activity was completely abolished by applying the AMPA-receptor antagonist NBQX (25 µM) and the NMDA-receptor antagonist D-AP5 (50 µM), suggesting that synaptic currents result solely from the activation of glutamate receptors (Fig. 5*d*). Further characterization of the synaptic activity revealed that TTX (1 µM) reduced the amplitude and frequency of excitatory postsynaptic currents by approximately 50%, suggesting that half of the events observed were evoked by action potential–

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dependent vesicle release and the remainder resulted from spontaneous vesicle release.

To characterize the neural network in an increasingly intact system, we sliced hCSs into 250 µm sections and performed acute whole-cell recordings (Fig. 5*e*). We found that 80% of neurons fire action potentials in response to depolarizing current steps from a holding potential of –65 mV. The large majority of neurons (86%) showed spontaneous synaptic activity that was reduced by kynurenic acid, a glutamate receptor blocker. Importantly, in response to extracellular electrical stimulation, we observed large-amplitude excitatory postsynaptic potentials (EPSPs; > 20 pA), demonstrating that cortical neurons in hCSs participate in network activity (Fig. 5*f*). To determine whether these synaptic responses are capable of driving spike firing in hCS neurons, we performed current-clamp recordings in which no holding current was applied while administering periodic electrical stimulation. Single spikes as well as burst events were observed after stimulation (Fig. 5*g*). Spontaneous spiking events were also observed in the absence of stimulation. These results demonstrate that an active network exists within hCSs that is capable of producing complex synaptic events associated with postsynaptic neuronal spike firing.

Summary

The hCS system provides a platform on which, together with deep and superficial cortical neurons, human astrocytes are generated from an identical genetic background. Astrocytes powerfully control synapse formation and function and are critical for proper neural development (Pfrieger and Barres, 1997; Ullian et al., 2001). In contrast to existing protocols for generating astrocytes from hiPSCs or by direct conversion, in the hCS system, the astrocytes are dispersed throughout the hCS and develop spontaneously, without the need for ciliary neurotrophic factor (CNTF), leukemia inhibitory factor (LIF), or serum, which are known activators of astrocytes. It is likely that the generation and close spatial integration of astrocytes inside hCSs mediate synaptogenic mechanisms that ultimately produce robust synaptic responses (Eroglu et al., 2009; Allen et al., 2012). In fact, neurons in the hCS are more transcriptionally and electrophysiologically mature than neurons generated through conventional methods. Finally, and importantly, hCSs are amenable to acute-slice physiology techniques, which have been extensively used to study circuits in animal brain slices. Excitatory neurons within acutely prepared slices are capable of complex synaptic events that result in postsynaptic neuronal spike firing, making hCSs an attractive model for investigating neural network activity in human cells. hCSs represent a versatile platform for patterning and specification of various neuronal and glial cell types as well as for designing large-scale drug screening *in vitro*. With the ability to derive hiPSCs from unique patient populations, this approach may prove a convenient and physiologically relevant platform for studying unique aspects of human corticogenesis as well as the pathophysiology of neuropsychiatric disorders, including synaptopathies and epilepsies.

Future Directions

Human cellular reprogramming technologies were introduced only recently, but their application to human disease modeling has been abundant. A PubMed search using the terms "induced pluripotent stem cells" and "disease" reveals more than 2300 articles. To date, *in vitro* models of brain diseases encompass both developmental and degenerative disorders and are based on the directed differentiation of neurons. Although cellular models for neuropsychiatric disorders were initially met with skepticism, a number of well-controlled studies to date indicate that we can surmount the variability associated with cellular reprogramming and differentiation *in vitro* and, more significantly, that these models can be utilized as a reliable platform for understanding disease pathogenesis. A major goal of this approach is the ability to run large-scale drug screening and perhaps even *in vitro* clinical trials for rare disorders, for which sufficient numbers of patients may not be available. It is becoming increasingly clear that drug responses within specific psychiatric conditions are quite variable. This observation has paved the way for another feasible application of iPSC technology: the development of iPSC-based assays that reliably predict drug responses in individuals. It remains to be seen whether large-scale, multidimensional cellular phenotyping in neurons from patients with idiopathic schizophrenia or autism spectrum disorders will yield novel operational parameters to improve our Kraepelian view of these disorders. The following is a series of issues to be addressed and future directions for the field of neural differentiation and *in vitro* disease modeling:

1. A comprehensive exploration of the functional properties and transcriptional signatures of forebrain neurons derived from multiple human and nonhuman primate species will provide novel evolutionary insights and guide the study of disorders of social cognition.

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2. A major missing element in our understanding of *in vitro* cellular reprogramming and human neural differentiation is a detailed mapping of epigenetics, imprinting, and X-inactivation phenomena that occur during these processes. This information will be essential for us to reliably address, in *in vitro* cellular models, neuropsychiatric conditions caused by disruptions in genes governed by such biological events.

3. Further studies are required to establish the actual age of neurons generated *in vitro* and to concretely identify their corresponding *in vivo* human developmental stage. In addition, better approaches for aging human neurons in the dish should be developed.

4. Integrated models of neuropsychiatric disease that address cell type–specific defects should be built to address complexities related to the intimate multicellular milieu existing in the brain. Specifically, these models should consider the relative contributions made by astrocytes, neurons, endothelial cells, microglia, or oligodendrocytes in modulating a specific cellular phenotype. Particularly in this setting, the requirement to carefully assess neuronal identity becomes apparent.

5. The field needs to create better infrastructure for sharing hiPSC clones among laboratories and institutions, and for collecting clinical details from patients whose cells are reprogrammed for subsequent cellular phenotype–clinical correlation studies.

6. Studies of neuropsychiatric disorders should be highly integrative, using complementary rodent disease models, human cellular models, and postmortem tissue.

7. With appropriate resources, studies of neurons derived from large populations of patients with idiopathic forms of psychiatric disease (i.e., enrolling hundreds to thousands of subjects) should be conducted to identify whether, with more statistical power, we can isolate disease subtypes, predict drug responses, or make clinical prognoses on the basis of cellular endophenotypes.

8. Human cellular models of disease are likely to reveal numerous cellular phenotypes for a given disorder. Some of these abnormalities will be core pathophysiological processes, whereas others will be homeostatic compensatory events or *in vitro* artifacts. We must develop novel approaches to determine the nature of identified cellular abnormalities so that we can efficiently target therapies to them.

9. The ultimate proof of the therapeutic potential of patient-derived neurons will come from demonstrating that a drug identified as correcting cellular phenotypes *in vitro* can result in clinical improvement in patients with a specific disorder. This outcome could arise from high-throughput *in vitro* screening with FDA-approved drugs and drug repurposing, or by running clinical trials in the dish for rare disorders for which the number of drugs to test surpasses the number of available patients.

Acknowledgments

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