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REVIEW

Emerging Human Pluripotent Stem Cell-Based Human–Animal Brain Chimeras for Advancing Disease Modeling and Cell Therapy for Neurological Disorders

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Abstract Human pluripotent stem cell (hPSC) models provide unprecedented opportunities to study human neurological disorders by recapitulating human-specific disease mechanisms. In particular, hPSC-based human–animal brain chimeras enable the study of human cell pathophysiology *in vivo*. In chimeric brains, human neural and immune cells can maintain human-specific features, undergo maturation, and functionally integrate into host brains, allowing scientists to study how human cells impact neural circuits and animal behaviors. The emerging human–animal brain chimeras hold promise for modeling human brain cells and their interactions in health and disease, elucidating the disease mechanism from molecular and cellular to circuit and behavioral levels, and testing the efficacy of cell therapy interventions. Here, we discuss recent advances in the generation and applications of using human–animal chimeric brain models for the study of neurological disorders, including disease modeling and cell therapy.

Keywords Human pluripotent stem cell · Human–animal chimera · Neurological disorder · Disease modeling · Cell therapy · Human neurons and glia · Microglia · Organoid

Introduction

Unraveling the underlying mechanisms and developing effective treatments for neurological disorders has been hindered by the species-specific disease phenotypes and mechanisms that are challenging to recapitulate in animals. Previous studies have shown significant species differences in the central nervous system (CNS) between humans and animals, spanning neurodevelopment to neurodegeneration in both health and disease [1–4]. During neurodevelopment, distinct species-specific features are reported in gene expression, progenitor cell types, and developmental time scales between humans and animals [2–4]. For example, the abundance of outer radial glia cells significantly increases during human brain development, leading to more neurons, a gyrencephalic shape, and a remarkable brain size, which does not occur to the same extent in animals [3, 4]. In addition, a prolonged duration of neuronal development is shown in humans compared with other species [4, 5]. Species differences also extend to various cell types in the CNS, including neurons [1, 6, 7], microglia [8–10], astrocytes [11–13], and oligodendroglial progenitor cells (OPCs)/oligodendrocytes [14]. For instance, human neurons can grow longer axons and form more synapses [1]. Interestingly, layer 5 neurons in humans have a lower density of ion channels and exhibit much lower voltage-gated K^+ and HCN conductances compared to animal neurons, which may allow human neurons to divert resources to other energy-intensive neuronal or circuit processes [6]. Distinct transcriptomic and functional differences are reported in human microglia, including differential expression of complement cascade genes, phagocytic functions, and susceptibility genes related to neurodegeneration [9, 10]. Notably, human-specific phenomena are evident in neurological disorders [15–17]. For instance, in Alzheimer's disease, human neurons show a unique vulnerability to cell death that is not seen in mice. As demonstrated by Strooper's group, in mice expressing high levels of human APP, human neurons display cell death similar to Alzheimer's disease patients, whereas endogenous mice neurons do not show neuronal cell death [17]. A follow-up study

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suggested strong upregulation of the long-noncoding RNA MEG3 in human neurons transplanted in an Alzheimer's disease mouse model. Overexpression of MEG3 results in necroptosis, and down-regulation and inhibition of necroptosis rescue neuronal loss in xenografted human neurons [18].

The species differences between humans and animals highlight the importance of utilizing human samples or cells for studying neurological disorders. However, access to functional human brain tissue is limited and it is difficult to manipulate directly. To circumvent these limitations, human pluripotent stem cells (hPSCs), which include human embryonic stem cells (hESCs) and induced pluripotent stem cells (iPSCs), have emerged as valuable tools. hPSCs can differentiate into most cell types in the human body, including cells in the CNS. The advent of hPSC-based models, including *in vitro* and *in vivo* models, provides unprecedented opportunities to study human neurological disorders using human cells.

Among these hPSC-derived models, the human–animal chimeric brain models offer a unique chance to study human cell pathophysiology in three-dimensional (3D) *in vivo* environments. In contrast, *in vitro,* hPSC-derived 2D monolayer culture and 3D brain organoid models are useful for examining basic aspects of neurodevelopment and neurological disorders, such as cell differentiation, migration, and neurogenesis. In addition, *in vitro* models serve as valuable tools for high-throughput genetic and drug screening. However, one major drawback of these models is their limited ability to replicate the complexity of synaptic connections and circuitry maturation, making them less suitable for evaluating the disrupted circuits and abnormal behaviors involved in diseases. On the other hand, within these chimeric brains, human cells (neuronal, glial, and immune cells) can recapitulate human-specific features, mature, and functionally integrate into host brains. This unique characteristic enables researchers to identify molecular, cellular, and circuit defects associated with neurological disorders, spanning neurodevelopment to neurodegeneration. As a result, chimeric brains provide valuable insights and facilitate the discovery of novel targets and the development of new therapeutics for patients. In this review, we discuss recent advances in the generation and applications of using human–animal chimeric brain models for studying neurological disorders, including disease modeling and cell therapy.

Types of Human–Animal Chimeric Brain Models

There are different ways to generate human–animal chimeric brain models, including the transplantation of hPSCs, hPSC- or fetal tissue-derived neural cells into animals at embryonic, fetal, neonatal, or postnatal stages. In these chimeric brains, human neural cells structurally and functionally integrate into the host brain and dynamically interact with host brain cells. Based on the time at which human cells are introduced into animals, there are four major types of human–animal chimeric brain models (Fig. 1). The first way, known as blastocyst complementation, involves microinjecting hPSCs into the pre-implantation blastocysts of genetically engineered animals (e.g., pigs) where functional genes necessary for the development of animal tissues of interest are disrupted. Subsequently, transferring these blastocysts into the uterus of a maternal surrogate can lead to human–animal chimerism across multiple organ systems, including neural cells [19, 20]. The second type is to transplant hPSC- or fetal human brain cell-derived stem cells/neural progenitor cells (NSCs/NPCs), or hPSCs, into prenatal animals [21–23]. For instance, the McKay group demonstrated that neural stems from human fetal brain cells transplanted into the telencephalic vesicle of embryonic day (E)17–E18 rats can differentiate into neurons, astrocytes, and oligodendrocytes, leading to widespread CNS chimerism in the host brain[23]. Another

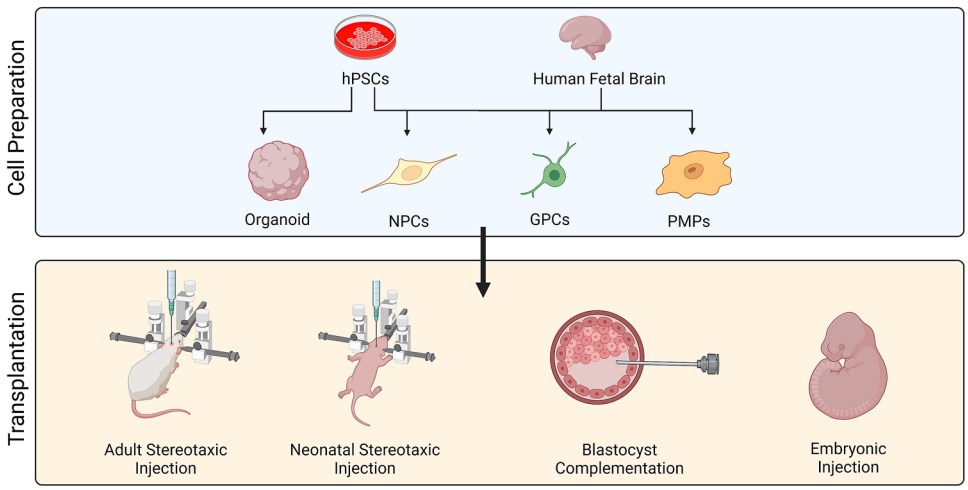

Fig. 1 Major types of human-animal chimeric brain models based on the cell sources and the time to introduce human cells into animals. Human pluripotent stem cells (hPSCs), neural progenitor cells (NPCs), glial progenitor cells (GPCs), and primitive macrophage progenitors (PMPs). The figure was generated by BioRender.

study by the Gage lab involved transplanting hESCs into the lateral ventricle of E14 mouse embryos, resulting in the differentiation of functional human neurons that successfully integrate into the adult mouse forebrain without forming teratomas [21]. The third method involves transplanting hPSC- or fetal/adult human brain cell-derived NSCs/NPCs or other neural cells, or hPSC-derived cerebral organoids, into the brains of neonatal (P0) to P3 animals [12, 24–28]. The Snyder group has successfully established the concept that engrafted human fetal brain cell-derived NSCs/NPCs in the germinal zone of neonatal mice can respond to developmental cues, utilize established migratory pathways, such as the rostral migratory stream, migrate to disseminated CNS regions, and differentiate into regionally appropriate neuronal cell types [28]. Another pioneering study from Goldman's group demonstrated that by transplanting fetal human glial progenitor cells (GPCs) into neonatal shiverer X rag2-/- immunodeficient mice, the engrafted cells can survive and divide for up to 1-year post-transplantation [27]. Notably, human GPCs differentiated into oligodendrocytes with substantial remyelination, and rescued the lethally hypomyelinated shiverer mouse [27]. The fourth method, transplanting fetal/adult human brain cell-derived NSCs/NPCs or other neural cells, or hPSC-derived cerebral organoids, into the brains of adult animals, can result in chimeras [29–31].

Human–animal chimeric brain models created through different methods offer both advantages and disadvantages. Typically, blastocysts and embryonic transplantation yield a high degree of chimerism. However, early transplantation poses significant ethical and technical challenges. Adult transplantation generally results in the lowest chimerism, but it offers a valuable opportunity to explore the strategy of using hPSC derivatives for cell therapy (or regenerative medicine) to treat neurological disorders. In contrast, neonatal transplantation allows human donor cells to respond to developmental cues and utilize migratory pathways in the developing brain, leading to a relatively high level of chimerism with fewer ethical and technical concerns. Thus, generating chimeras at the neonatal and adult stages is currently considered more feasible for advanced biomedical research. Therefore, the subsequent sections will focus on chimeras generated through neonatal and adult transplantation. We will discuss the advances in using human–animal chimeric brain models based on the engrafted cell types (Fig. 2) for studying neurological disorders, including disease modeling and cell therapy.

Human–Animal Neuronal Chimeric Brain Model

As major components of the CNS, neurons play pivotal roles in various neurological disorders [32], where dysfunction or impairment of neurons often contributes to disease progression. For instance, autism spectrum disorders have been linked to an imbalance between excitatory and inhibitory neurons [33], while severe neurodegeneration is a hallmark of advanced Alzheimer's disease [34]. To gain a deeper understanding of the mechanisms behind neurological diseases and to explore potential cures, it is crucial to develop

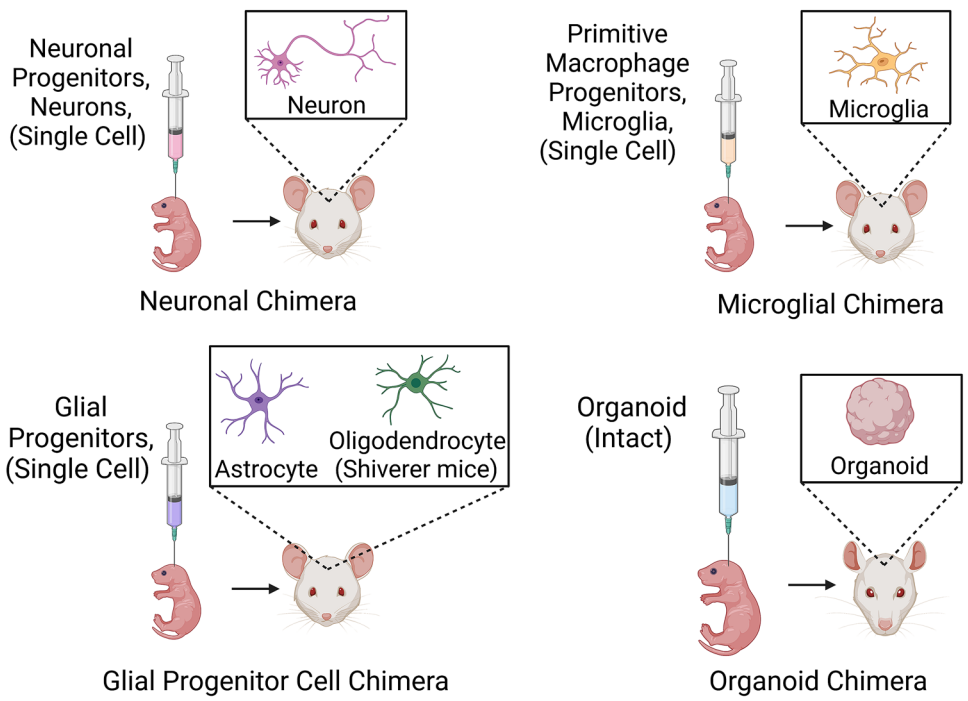

Fig. 2 Human pluripotent stem cell-based human-animal chimeric brain models through neonatal and adult transplantation based on the engrafted cell types. The chimeras include neuronal, microglial, and glial (astroglial and oligodendroglial) chimeras in mice, as well as organoid chimeras xenografted in neonatal rat and adult mice. The figure was generated by BioRender.

better models of human neurons that accurately represent human physiological conditions.

hiPSC-derived neural progenitor cells (NPCs) have shown promise in differentiating into neurons [35–37], and their transplantation into neonatal mouse brains has yielded substantial results. Six to 13 months after transplantation, xenografted human NPCs differentiate and mature into neurons, widely distributed in the mouse brain, such as the cerebral cortex and hippocampus [38]. In a recent study, co-transplanting NPCs and blood vessel-forming vascular cells significantly improved graft size in a mouse model of stroke when compared to NPC transplant alone, suggesting the potential for more advanced and complex chimeric brain models for research purposes [39].

Human–animal neuronal chimeric brain models have been instrumental in studying various neurological disorders, including disease modeling and cell therapy (Table 1). To model human neurological disorders, neuronal chimeras have been developed. For instance, transplanting iPSC-derived cortical neuronal precursors into Alzheimer's disease mouse models successfully recapitulated the expression of three-repeat (3R) and four-repeat (4R) tau splice forms, a phenomenon unique to humans [17]. Genome-wide expression analysis revealed upregulation of genes associated with myelination and downregulation of genes related to memory, cognition, synaptic transmission, and neuron projection, providing a valuable tool to study human-specific pathologies in Alzheimer's disease [17]. Moreover, transplantation of Down syndrome (DS) iPSC-derived interneuron progenitor cells into neonatal immunodeficient mice can lead to the generation of DS chimeric mice with an overabundance of interneurons and impaired learning and memory at 6 months post-transplantation, compared to control chimeras that received donor cells differentiated from control iPSCs. Notably, chimeras that engrafted DS interneuron progenitor cells with inhibited OLIG2 expression by shRNA can reverse the overproduction of interneurons and improve animal behavioral performance [40]. Another study transplanted DS iPSC-derived GABAergic neurons into the medial septum of 8 to 10-week-old SCID mice. GABAergic DS neurons exhibited smaller soma size, fewer processes, and impaired migration compared to control groups [41]. In addition, transplanting human neurons into the mouse forebrain successfully recapitulated the augmentation of excitatory transmission found in autism [42]. These results demonstrated that human–animal neuronal chimeric brain models enable modeling of the pathophysiology of human neurons in neurological disorders and unravel the mechanisms behind neuronal death.

On the other hand, human–animal chimeric brain models hold the promise of testing human neurons as cell therapy to treat neurological disorders. Engraftment of human neural stem cells into Huntington's disease mouse models resulted in improved motor deficits and rescued synaptic alterations [43]. Furthermore, a reduction in aberrant mHTT protein accumulation and brain-derived neurotrophic factor levels was found in the host brain, indicating the potential therapeutic benefits of this approach [43]. More recent studies have shown that transplantation of hiPSC-derived NPCs into Huntington's disease mouse models can delay disease progression and exhibit neuroprotective effects, holding promise for stem cell-based cell therapy [44]. Notably, hECS-derived midbrain dopamine (mDA) neurons have been extensively grafted into Parkinson's disease mouse neural circuitry [45]. These transplanted mDA neurons exhibited characteristics resembling the A9 subtype of DA neurons and successfully restored motor function in mice. These findings also underscored the importance of the specific cell type in facilitating functional synaptic inputs, emphasizing the capacity of human pluripotent stem cell-derived neuron subtypes for targeted circuit repair and functional restoration [45]. This research is a promising step toward potential therapeutic applications in treating Parkinson's disease and other neurological disorders related to dopaminergic dysfunction. In addition, it is reasonable to assume that neurotrophic factors produced by implanted neurons can impact the pathologies and behaviors of the host.

Beyond disease modeling and cell therapy, neuronal chimeras offer valuable insights into neuronal development. Transplantation of hiPSC-derived cortical pyramidal neurons into the mouse cortex revealed single-cell integration, unveiling a coordinated developmental roadmap for human cortical neurons [26]. These engrafted human neurons exhibited a prolonged developmental timeline, displaying decorrelated, tuned responses to visual stimuli, confirming circuit-level integration [26]. Further investigation demonstrated slower mitochondrial development and lower activity in oxidative phosphorylation in human neurons, suggesting a potential role for mitochondria in regulating the pace of neuronal development [5].

Previous studies demonstrated that engrafted human neurons in neonatal and adult brains can mature, form synapses with host neurons, and functionally integrate into the host brain, allowing us to assess the effects of human neurons on animal behaviors [7, 24]. Multiple techniques, including morphology, electrophysiology, and rabies-mediated tracing, can be applied to confirm the formation of synapses and neural circuit integration [43, 45]. For instance, for neonatal transplantation, our studies show that hiPSC-derived human neurons form synapses with host neurons and exhibit electrophysiological properties, including action potentials and spontaneous postsynaptic currents and spontaneous postsynaptic potentials 6 months post-transplantation, demonstrating their maturity and functional integration into the host brain. Furthermore, behavioral tests revealed that DS chimeric mice exhibit impaired learning and memory compared with control chimeras [25]. In addition, in the adult

Table 1 Human-Animal Brain Chimeric brain models for disease modeling and cell therapy

Cell type	Disease	Injected cell	Host	Injection time point	Injection site	Outcomes	References
Neuron	AD	hPSCs- cortical derived progenitors and neurons	APP PS1 tg/wt NOD-SCID (AD mice), APP PS1 wt/wt NOD-SCID (WT mice)	p0	Frontal cortex	Transplanted cells matured and functionally integrated into host brains expressed 3R/4R splice forms Human-specific vulnerability to AD pathology	Espuny-Camacho *et al.* [17]
	PD	hESC-derived mDA neurons	SCID mice	Adult	Left nigra/left striatum	Transplanted cells extensively grafted into host circuitry Graft location was critical for presynaptic inputs Human mDA neurons displayed A9 characteristics and restored motor function	Xiong *et al.* [45]
	HD	hESC-derived hNSCs	R6/2 mice	5-weeks old	Striatum	· hNSC transplantation improved motor deficits, synaptic alterations, and integrated into host neural circuits	Reidling *et al.* [43]
		hiPSC-derived NPCs	YAC128 HD mice	6 months adult mice	Striatum	NPCs exhibited multiple differentiation potential DARPP-32 expression restoration after transplantation, improved motor and cognitive function in transplanted mice	Park *et al.* [44]
	DS	hiPSC-derived DS GABAergic progenitors	SCID mice	8 to 10 weeks old	Medial septum	DS GABAergic neurons exhibited smaller soma size and fewer processes DS GABAergic neurons had impaired migration	Huo *et al.* [41]
		hiPSC-derived DS NPCs	rag1-/- mice	p0	Hippocampus and cortex	DS NPCs gave rise to GABAergic neurons, causing impaired recognition memory in the host Knockdown of OLIG2 largely reversed abnormal gene expression and reduced Interneuron production, improved behavioral deficits in DS chimeric mice	Xu *et al.* [40]
	Autism	hPSC-derived neurons	rag2-/- mice	p0-p3	Hippocampus and cortex	Human neurons with disease mutations augmented excitatory transmission	Wang *et al.* [42]
	Neurodevelopment	hESC-derived cortical neurons	NOD-SCID or rag2-/- mice	p0-p1	Lateral ventricle	Transplanted cells exhibited a prolonged developmental timeline Functionally integrated into host neural circuits.	Linaro *et al.* [7]
		hESC-derived cortical neurons	NOD-SCID or rag2-/- mice	p0-p2	Lateral ventricle	· Mitochondria are regulators of the pace of neuronal development underlying Human-specific brain neoteny.	Iwata *et al.* [5]
Astrocyte	AD	hiPSC-derived astrocyte progenitor cells	*APP PS1* tg/wt NOD-SCID (AD mice), *APP PS1* wt/wt NOD-SCID (WT mice)	p0-p4	Forebrain	Transplanted cells differentiated into astrocytes retaining human-specific morphologies Transplanted cells undergo morphological changes in response to amyloid beta Plaques	Preman *et al.* [56]

Table 1 (continued)

Cell type	Disease	Injected cell	Host	Injection time point	Injection site	Outcomes	References
	HD	hESCs-derived or mHTT-transduced fetal hGPCs	R6/2 x rag1-/-, rag1-/-	p1	Striatum	HD GPCs impaired motor learning in normal mice, striatal neurons exhibited neuronal input resistance and excitability Replacement of diseased glia with normal glia substantially slowed the disease progression and increased survival of R6/2 mice	Benraiss *et al.* [53]
		hESC-derived hGPCs (HD or WT)	rag1-/- mice	p0-p2; 9 months adult mice	Striatum	hGPCs engrafted in adulthood competitively replaced neonatally transplanted HD/WT astrocytes in an age-dependent manner WT hGPCs acquired a YAP1/MYC/E2F-defined dominant competitor phenotype when interacting with the host glia	Vieira *et al.* [54]
	JC virus infection	human fetuses isolated bipotential GPCs	rag2-/-/Mbp$^{shi/shi}$, rag1-/-	p1	Forebrain subcortex	Astrocyte infection is sufficient for JCV spread, JCV infection is associated with progressive mutation of JCV capsid protein VP1 A model for studying human-selective virus in vivo	Kondo *et al.* [58]
	DS	hiPSCs-derived Di-DS3 and Tri-DS3 astroglia	rag1-/- immunodeficient mice	p0	Lateral ventricles (LVs)	Transplanted cells integrated into host tissues and were large, retaining human astroglia features DS astroglia exhibit higher levels of reactive oxygen species and lower levels of synaptogenic molecules	Chen *et al.* [55]
	Schizophrenia	hiPSC-derived SCZ GPCs	shiverer x rag2-/- mice, rag1-/- mice	p1-p2	Rostral and caudal corpus callosum	Transplanted cells exhibited premature migration into the cortex, reduced white matter expansion, and hypomyelination Delayed astrocyte differentiation of human cells in shiverer mice	Windrem *et al.* [66]
	Neurodevelopment	Human fetus isolated GPCs	rag1-/- or rag2-/- immunodeficient mice	p1	Forebrain	Transplanted human cells were larger in size, had longer processes, and encompassed more synapses Human cell gap-junctions coupled to host astroglia enhanced LTP and learning in chimeric mice	Han *et al.* [12]
Oligodendrocyte	Demyelination	Fetal OPCs and adult OPCs	Shiverer mice	p1	Corpus callosum	Both fetal and adult OPCs myelinated the host brain Adult OPCs generated oligodendrocytes more efficiently than fetal OPCs	Windrem *et al.* [61]
		Fetal hGPCs	Shiverer x rag2-/- mice	p1	Forebrain subcortex	Cerebral chimeras with human white matter glial composition Prolonged survival with the progressive resolution of neurological deficits in transplanted mice	Windrem *et al.* [27]
		Fetal hGPCs	Shiverer x rag2-/- mice, rag1-/- mice	p1; adult	Corpus callosum	hGPCs broadly disperse and differentiate into oligodendrocytes in adult hosts, improving both host callosal conduction and ambulation Transplanted hGPCs remyelinate the CNS after cuprizone-induced demyelination both in neonatal transplanted mice and adults transplanted after infectious	Windrem *et al.* [63]

Table 1 (continued)

Cell type	Disease	Injected cell	Host	Injection time point	Injection site	Outcomes	References
		hiPSC-derived oligodendrocytes	Shiverer x rag2-/- mice	p1; adult	Corpus callosum; spinal cord	iPSC-derived oligodendrocytes are capable of remyelination host CNS during development and after demyelination	Ehrlich *et al.* [65]
		hiPSC-derived OPCs	Shiverer x rag2-/- mice	p1	Corpus callosum	hiPSC-derived OPCs remyelinated the host brain, substantially increasing the host survival	Wang *et al.* [62]
	Spinal cord injury	hPSC-derived OPCs	NOD-SCID mice	Adult	9 days after the spinal cord injury in the lesion center	Grafted OPCs formed myelin sheathes and diffused into white matter Enhanced functional recovery following SCI in transplanted mice	Kawabata *et al.* [64]
Microglia	AD	hiPSCs-derived hematopoietic progenitor cells	MITRG mice	p1	Lateral ventricles and overlying anterior cortex, posterior cortex	Transplanted progenitor cells differentiate into microglia in the host brain in a context-dependent manner, retaining the human microglial gene signature Transplanted microglia responded to Aβ plaques, and exhibited robust human-specific Transcriptional profiles such as CD33	Hasselmann *et al.* [85]
		hESC-derived microglia	rag2-/- mice	p4	Frontal cortex	Transplanted cells matured into human microglia Microglia changed from a homeostatic state towards a cytokine response state in which multiple inflammatory cytokines and chemokines are highly expressed.	Mancuso *et al.* [84]
	DS	hiPSC-derived DS PMPs	rag2-/- mice	p0	Hippocampus and cortex	DS microglia showed excessive synaptic pruning, exhibited cellular senescence and elevated type-I-interferon signaling in response to human pathological tau IFNAR knockdown rescued the DS microglia disease phenotype both during Development and in response to pathological tau	Jin *et al.* [86]
	Demyelination	hiPSC-derived PMPs	rag2-/- mice	p1	Hippocampus and cortex	Xenografted microglia retained human microglia identity Upregulation of CD74 and SPP1 in response to cuprizone-induced demyelination	Xu *et al.* [25]

brain, transplanted hESC-derived mDA neurons successfully integrate into the host's neuronal circuits, and repair nigro-striatal circuit both anatomically and functionally, indicating the feasibility of neural-circuit integration and re-construction by transplanting hPSC-derived NPCs [45].

These findings collectively highlight the importance of neuronal chimeras as a powerful tool for studying neurological disorders and unraveling the complexities of neuronal development.

Human–Animal Astroglial Chimeric Brain Model

Astrocytes are starlike cells that reside in the CNS and possess elaborate processes, playing essential roles during both brain development and adulthood [46, 47]. In the developing brain, astrocytes secrete different molecules that facilitate the formation and maturation of neural circuits [48]. In the adult brain, they assume critical responsibilities, including the maintenance of an appropriate chemical environment, neurotransmitter recycling at the synaptic cleft, and participation in the construction of the blood–brain barrier [49]. Perturbations in astrocyte function have been reported in numerous neurological diseases, such as Alzheimer's disease, Huntington's disease, Parkinson's disease, and amyotrophic lateral sclerosis [50–52]. As such, a comprehensive exploration of astrocytic characteristics assumes paramount importance in advancing our understanding of neurological disorders. However, the differences between human and rodent astrocytes, for example, the size of the cell, process number, and length, and the number of synapses each cell encompasses [13], introduce noteworthy limitations in existing animal models, thereby amplifying the challenges encountered in deciphering the underlying mechanisms of the complex pathobiology.

Human–mouse astroglial chimeric brain models have emerged as valuable tools in the field of neurobiology, offering a unique chance to decipher the roles of human astrocytes in human disease pathogenesis and potential therapeutic avenues. For instance, when hESC-derived GPCs expressing mutant Huntingtin (*mHTT*) are transplanted into the normal neonatal mouse brain, multiple disease phenotypes such as impaired motor learning and hyperexcitation of striatal neurons show up, indicating a causal role of glia pathology in Huntington's disease [53]. In adult YAC128 Huntington's disease mice, transplantation of human NPCs restores DARPP-32 expression in the host brain [44]. Recently, a study transplanted wild-type human GPCs (WT-hGPCs) into adult mice that had previously received neonatal injections of mHTT-expressing hGPCs. The transplanted WT-hGPCs outcompeted and replaced the diseased glial cells. WT hGPCs acquired a YAP1/MYC/E2F-defined dominant competitor phenotype when interacting with host glia, highlighting the promise of intracerebral GPC delivery as a potential cell therapy for neurological disorders [54]. While these findings are promising, further experiments and clinical trials are imperative to fully understand the therapeutic implications.

Chimeric brain models are a promising tool for studying neurological disorders, such as DS and Alzheimer's disease, offering insights into astrocyte functions and their responses in these conditions. In the context of DS, researchers used human-induced pluripotent stem cells (hiPSCs) to generate DS astroglia, which was then transplanted into mouse brains [55]. Notably, the engrafted DS astrocytes displayed larger cell sizes and longer processes, closely resembling the properties of adult human astroglia *in vivo*. However, unlike the isogenic control counterpart, the transplanted DS astrocytes fail to induce neurogenesis, providing valuable insights into the specific functions of astrocytes in DS [55]. In addition, hiPSC-derived astrocyte progenitors engrafted into neonatal Alzheimer's mouse brains are capable of maturing and functionally integrating into the host brain [56]. Notably, when confronted with amyloid beta plaques, transplanted astrocytes exhibit morphological changes, offering a reliable model for studying the role of astrocytes in Alzheimer's disease within the context of the human genetic background [56].

Transplantation of GPCs isolated from human fetuses into neonatal rag1-/- or rag2-/- mice resulted in high proportions of both hGPCs and astrocytes in the host brain. The engrafted cells retained the characteristic size and pleomorphism of hominid astroglia, and their presence notably improved activity-dependent plasticity and learning in the mice [12]. In addition, by transplanting GPCs into neonatal mouse cerebral cortex, Padmashri et al. also successfully recapitulated human interlaminar astrocytes [57], providing a platform for investigating neuron-glial interactions, which often undergo significant functional alterations in various neurological disorders. Moreover, chimeric brain models hold considerable potential in the study of human-selective infectious diseases, such as the John Cunningham virus (JC virus, JCV), which lacks suitable animal models for studying disease progression. By transplanting human fetal-derived bipotential GPCs into immunodeficient shiverer mice, researchers were able to fill this void and gain insights into virus spreading through astrocyte infection [58]. Such models offer unprecedented opportunities to investigate the pathogenesis of human-specific infections and develop potential therapeutic strategies.

Human–Animal Oligodendroglial Chimeric Brain Model

Oligodendrocytes are crucial for fast signal transmission in the CNS, and recent research highlights their potential

in treating demyelinating conditions through the transplantation of hiPSC-derived oligodendrocyte progenitor cells. Oligodendrocytes form a laminated, lipid-rich wrapping around axons known as myelin, facilitating rapid conduction of action potentials [59]. Demyelination, which can result from disease or injury, leads to slowed and interrupted electrical signal transduction [60]. Despite their essential function in ensuring efficient electrical transmission, the significance of oligodendrocyte dysfunction in the progression of neurodegenerative diseases and psychiatric disorders has long been underestimated. However, recent years have seen a growing body of evidence demonstrating the potential of transplantation of hiPSC-derived OPCs as a promising treatment for demyelination in various conditions, including spinal cord injury (SCI), leukodystrophies, and amyotrophic lateral sclerosis.

Pioneering work by Windrem et al. in 2004 demonstrated that both fetal and adult OPCs can re-myelinate the shiverer mouse brain when transplanted into the corpus callosum of newborn mice [61]. Subsequent studies utilizing hiPSC-derived OPCs in immunodeficient shiverer mice demonstrated even faster and more efficient myelination compared to the use of fetal OPCs [62]. Neonatally engrafted OPCs exhibited widespread distribution and remyelination, significantly prolonging the survival of congenitally hypomyelinated host mice, which would typically not survive past 18–21 weeks after birth [27]. In another study, OPCs were transplanted into multiple sites in the corpus callosum and presumptive cerebellar peduncle of shiverer x rag2-/- mice, resulting in notable resolution of neurological deficits [63]. Mice surviving 130–150 days post-transplantation showed improved ambulation and a lower frequency of seizures. The transplantation of OPCs successfully restored the nodes of Ranvier, normalized transcallosal conduction velocities, and achieved relatively complete myelination of most axons. Moreover, xenografted hGPCs exhibited remyelination in response to cuprizone-induced demyelination, accompanied by significant changes in signaling pathways related to lipid uptake, Notch signaling, and cell proliferation [63].

Similar encouraging results were reported in studies conducted in adult mice. In a model of adult SCI using NOD-SCID (nonobese diabetic-severe combined immunodeficient) mice, OPCs were transplanted into the lesion epicenter [64]. These engrafted OPCs successfully differentiated into oligodendrocytes and migrated extensively into the white matter of the injured spinal cord, forming thick myelin sheaths. As a result, the transplanted group showed significantly enhanced recovery of motor function, as assessed through behavioral tests and electrophysiological examinations [64]. In addition, in adult shiverer mice, hiPSC-derived oligodendrocytes grafted into the dorsal funiculus are widely distributed throughout the host brain, leading to successful remyelination [65]. Similarly, transplantation of hGPCs in a cuprizone-induced demyelination model also demonstrated successful remyelination, accompanied by transcriptional profile alterations that indicated the directed differentiation of GPCs into oligodendrocytes and myelin formation [63].

Considering disease modeling, the engraftment of hiPSC-derived GPCs from individuals with schizophrenia into mouse brains reveals insights into the role of glia in the disorder. The transplanted cells exhibit altered migration patterns, reduced white matter expansion, hypomyelination, and delayed astrocyte differentiation, accompanied by behavioral abnormalities and reduced prepulse inhibition [66].

While these findings hold promise for the development of potential therapeutic approaches, further research is required to delve into the molecular mechanisms underlying the OPC/GPC-mediated remyelination process and to gain a deeper understanding of oligodendrocyte dysfunction in various neurological disorders. Such investigations will be instrumental in advancing our knowledge and paving the way for more effective treatments targeting demyelination-related conditions.

Human–Animal Microglial Chimeric Brain Model

Microglia, the resident immune cells of the CNS, play a crucial role in maintaining the physiological environment by clearing cellular debris and infectious agents [67, 68]. In addition, they secrete signaling molecules, such as cytokines, to regulate inflammation [69]. Traditionally, microglial states were classified as either "resting" or "activated" based on their ramified or amoeboid morphology [70]. However, with advances in sequencing technology, it has become evident that microglial states are more intricate and nuanced, requiring more specific classification to better comprehend microglial biology [71].

In the context of neurodegenerative diseases, microglia can act as a double-edged sword. In the early stages of disease progression, microglia contribute to beneficial debris clearance [72]. However, chronic and prolonged activation of microglia leads to neuroinflammation, which is detrimental to neurological diseases [73]. The spreading of abnormal tau protein [74] or α-synuclein [75] in these conditions can be attributed to microglia, as they phagocytose these proteins from other neurons and deposit them into healthy ones. Interestingly, many of the >70 loci associated with Alzheimer's disease that have been revealed through genome-wide association studies are highly expressed by microglia [76]. Recent data from single-cell and nuclear RNA sequencing has shed light on the specific responses and stages of disease-associated microglia in various disease conditions [77]. These analyses have also identified several microglia-specific genes, including Triggering Receptor Expressed on Myeloid Cells 2 (TREM2) and Membrane-spanning

4-domains subfamily A (MS4A), whose expression is regulated during disease progression [78, 79].

One challenging issue in studying the functionalities of genes related to microglia is the significant species differences between animals and humans [80]. This poses a dilemma for animal models as they can model some pathologies but fail to recapitulate essential features of human microglia, potentially hindering drug development and leading to higher chances of failure in clinical trials due to species differences. Isolating microglia from the human brain can also be a challenge as they undergo rapid and significant changes after isolation, and the availability of human brain tissue is limited [9]. While *in vitro* culturing of microglia derived from hiPSCs can partially recapitulate some properties, the maturation of these microglia may exhibit high variability which may be due to the lack of a physiological environment [81]. These obstacles have led researchers to explore human microglial chimeric mouse brain models as a promising alternative. These models combine microglia with a human genetic background and provide a physiological environment through the rodent host.

Transplanting hiPSC-derived microglia into specialized mouse models has successfully created chimeric brain models that mimic human microglial behavior and responses, offering valuable insights into neurological disorders and disease mechanisms. Microglial survival depends on the cytokine CSF1 [82, 83] and rodent CSF1 cannot support the survival of human microglia. Thus, transplanting hiPSC-derived microglia or microglia progenitor cells into *Rag2-/- Il2γ-/- hCSF1KI* or MITRG mice, which have a human version of hCSF1, successfully constructs such a model [21, 25, 84, 85]. Transplanted cells differentiate into different subtypes of CNS macrophage in a context-dependent manner and recapitulate the human microglial transcriptomic signature [21, 25, 84, 85]. These engrafted human microglia are functional in the chimeras. For instance, transplanted human microglia display the ability to respond to acute insults and exhibit upregulation of TREM2 expression in response to amyloid pathology [85]. Furthermore, the study demonstrated that transplanted human microglia express human-specific Alzheimer's disease risk genes, and their response to oligomeric Aβ differs from that of mouse microglia, such as the expression of CD33 [85]. hiPSC-derived primitive macrophage progenitors (PMPs) injected into neonatal mice brains can migrate along the corpus callosum into various brain regions during brain development, differentiate into functional mature microglia, and respond to cuprizone-induced demyelination [25]. Another significant advantage of using hiPSC-derived microglia is the flexibility in cell sources, allowing for studies using microglia derived from both healthy individuals and patients, offering insights into disease mechanisms. For instance, using microglial chimeric brains, a study has demonstrated that DS microglia exhibit excessive synaptic pruning during development and induce senescence when challenged with pathological tau, which is dependent on type-I interferon (IFN-I) signaling [86]. All these studies have convincingly demonstrated that microglia retain their identities in the host brain, making chimeric brain models a powerful tool for understanding microglial biology and their role in various neurological disorders.

Microglia are garnering increasing attention as a potential therapeutic target for cell therapy due to their critical role in various neurological disorders. Several studies have successfully achieved CNS-wide microglial replacement to alleviate pathologies [87, 88]. Transplanting hematopoietic cells into a TREM2 mutant mouse model rescued the loss of microglial function caused by TREM2 mutation and xenografted cells replaced mouse microglia. However, to enhance the engraftment of microglia into the adult mouse brain, it may be necessary to reduce the presence of resident mouse microglia [89]. This reduction can be accomplished through the administration of compounds like PLX3397 or PLX5622 [90, 91], as well as other methods, including radiation or chemotherapy [87, 92]. In a recent breakthrough, engineered human microglia carrying a CSF1R mutation that confers resistance to CSF1R inhibitors exhibited extensive dispersion throughout the mouse brain [88]. This exciting result opens up new possibilities for microglial replacement in patients. Nevertheless, further research is needed to explore additional avenues for utilizing microglia as a potential cell therapy in the treatment of neurological disorders.

Human–Animal Organoid Chimeric Brain Model

Compared to traditional hPSC-based two-dimensional cell cultures, the development of three-dimensional organoids has revolutionized *in vitro* cell research, providing a more realistic representation of cell–cell, and cell–cell matrix interactions. hPSC-based cerebral organoids, which consist of multiple cell types in the CNS organized in a structured manner, offer a remarkable resemblance to the organs of interest in terms of gene expression, protein expression, and cellular architecture [93]. Recent advances in organoid research have led to attempts to transplant whole organoids into the brains of rodents, resulting in functional integration of the organoids into the host neural circuits [94].

When transplanted into the brains of adult NOD-SCID immunodeficient mice, organoids demonstrate progressive differentiation and extensive axonal growth. Growth of the vascular network within the transplanted organoids also occurs, indicating the crucial role of the blood supply for their survival. Intriguingly, two-photon imaging of the grafts revealed neuronal activity, and as the organoids underwent longer maturation in the host brain, they exhibited more active and coordinated neuronal networks [95]. Similarly,

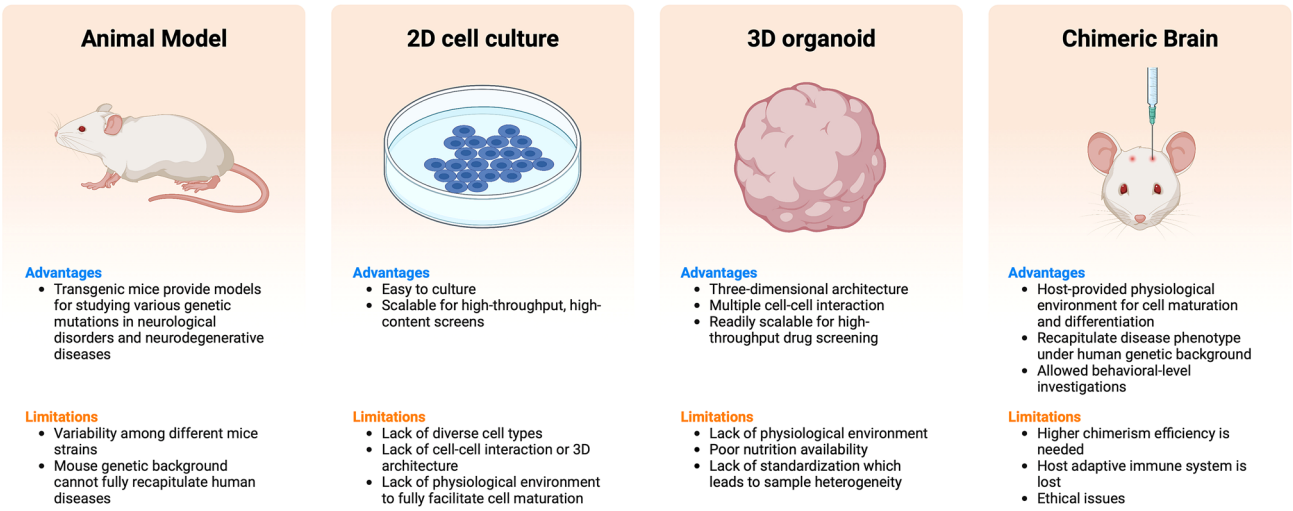

Fig. 3 Comparisons between different types of models for neurological disorders. Advantages and limitations are listed for the traditional animal model and human pluripotent stem cell-based 2D cell culture, 3D organoid, and the chimeric brain model.

hPSC-derived cortical organoids transplanted into neonatal rat brains showcased integration into the host neural circuits [24]. The transplanted organoids effectively responded to thalamocortical and corticocortical inputs, as well as optogenetic stimulation, underscoring their potential to provide a powerful approach to studying neurological disorders from a circuit-level perspective. Recently, hPSC-derived cortical forebrain organoids colonized by erythromyeloid progenitors were transplanted into the retrosplenial cortex of immunodeficient NPD.Cg-Prkdc^{scid}/J mice. This transplantation allowed researchers to explore human microglial function within a physiologically relevant human brain environment. The mature human microglia present in the organoids were found to actively surveil the tissue, respond to local injuries, and react to inflammatory signals [29]. These models greatly facilitate disease modeling studies, offering invaluable insights into the complex neural interactions underlying various neurological conditions.

Comparison of Human PSC-based 2D Culture, 3D Brain Organoids, and Human–Mouse Chimeric Brain Models

In the pursuit of cures for neurological disorders, it is essential to comprehend the underlying mechanisms of disease progression and various pathologies. While animal models have provided some insights, their limited applicability due to species differences has posed challenges [96] (Fig. 3). The advent of hPSCs, particularly patient-derived iPSCs, has revolutionized disease modeling. The ability of hPSCs to differentiate into various cell types has enabled the modeling of disease progression. Initially, most disease-modeling efforts focused on two-dimensional cell cultures. Although convenient for high-throughput screening, the lack of cell diversity and cell–cell interactions limited their utility in uncovering cellular phenotype changes under disease conditions [93]. The emergence of hPSC-derived 3D organoids, which contain multiple neural cell types and exhibit preliminary structural features of the brain [97], partially addressed these limitations. Various organoids targeting different disorders have been developed, significantly enhancing our understanding of diseases such as Alzheimer's disease, Parkinson's disease, frontotemporal dementia, neurodevelopmental disorders, and neural toxicity [93, 98]. Research is continually refining organoids, aiming to better recapitulate the pathophysiology of human brain environments by incorporating different cell types to mimic complex cell–cell interactions [99]. However, there remains a maximum capacity for organoid models, and the intricate physiological environment of the brain cannot be fully replicated *in vitro*. Microglia, in particular, with their sensitivity to the environment and complex responses under different disease conditions, require a better model to understand the biology of neurological disorders and the interplay of cells underlying disease phenotypes.

The development of human–mouse chimeric brain models has significantly addressed the challenges of mimicking physiological conditions. The rodent brain provides a natural physiological environment for human neural and immune cells individually or within organoids, to mature and differentiate, faithfully recapitulating cellular morphology, transcriptome profiles, and responses to acute and chronic insults to the best possible extent. Neonatally engrafted chimeric brains are suitable for studying human-specific neurodevelopment and neurological disorders [100–103], while chimeric brains constructed in adult rodents hold promise for

advancing cell therapy and disease modeling [104]. Numerous studies have yielded substantial outcomes, confirming the potential of chimeric mouse brain models in neuroscience research. Despite ethical concerns about the potential humanization of animals, human–animal brain chimeras generated from neonatal and adult transplantation, whether by hPSC-derived neural cells or organoids, are unlikely to develop ''human-like'' characteristics, such as self-awareness or advanced cognitive capacities [94, 105]. However, the ethical question of augmenting discrete brain functions in chimeras, such as learning and memory, remains relevant as reviewed by others [94]. By continuing to develop these models in a controlled and transparent manner, chimeric brain models can become an invaluable toolset for deepening our understanding of human brain development and pathobiology.

Conclusions and Perspectives

The Characterization and Application of Human–Animal Brain Chimeras

In general, the human–animal brain chimeras offer exceptional opportunities for modeling human neurodevelopment, investigating neurological disorders, and advancing cell therapy (Fig. 4). The chimeras enable the exploration of unique questions, such as: (1) modeling human-specific developmental milestones, maturation, and the aging of human neural and immune cells (such as microglia) in the long-term within 3D intact brain environments; (2) understanding how human neural cells integrate into neural circuits; (3) deciphering how risk genes, single-nucleotide polymorphisms, gene-environment interactions, and the patient's disease conditions influence the cellular functions of human neural and immune cells, as well as their impact on neural circuitry formation, synaptic plasticity, and animal behaviors; (4) investigating whether manipulation of specific genes or signaling pathways in human neural cells can rescue disease phenotypes; (5) evaluating the efficacy of candidate drugs in treating neurological disorders and decreasing species difference-caused translational failures; and (6) assessing the potential of utilizing healthy or engineered human neural and immune cells as cell therapy for treating neurological disorders. Multiple approaches, including CRISPR gene-editing, multiple omics, immunohistochemistry, electrophysiology, two-photon living imaging, and behavioral tests, can be applied to comprehensively characterize human cells *in vivo* across molecular, cellular, circuit, and behavioral levels.

In addition to their utility in disease modeling and elucidating the mechanisms underlying neurological disorders, one of the most important applications of chimeric brain models lies in providing crucial safety and feasibility assessments for the eventual implementation of iPSC-derived progenitor cells as a potential clinical cell therapy. As discussed above, previous investigations have convincingly demonstrated the feasibility of intracerebral delivery of iPSC-derived progenitor cells, with transplanted cells ameliorating pathologies and prolonging the survival of host animals, thereby affirming the safety profile of iPSC-derived progenitor cell transplantation. Furthermore, multiple ongoing clinical trials have yielded promising outcomes in this regard. For instance, the transplantation of patient iPSC-derived midbrain dopaminergic progenitor cells into the putamen of Parkinson's disease patients resulted in post-surgical cell survival and clinical assessments indicating symptom stabilization or improvement over an 18 to 24-month follow-up period [106]. Significantly, these implanted cells exhibited no discernible immunogenicity in mouse models, thereby offering a promising method for future clinical applications [106]. Another clinical trial focused on the transplantation of hESC-derived retinal pigment epithelium, which successfully mitigated visual acuity loss in two patients over a 12-month period. Remarkably, this trial only implemented localized immunosuppression, suggesting the potential for broader applications of such transplantation procedures [107]. Furthermore, Kyoto University is in the planning stages of clinical trials involving the transplantation of iPSC-derived neural stem/progenitor cells (NS/PCs) into subacute SCI patients, with the study protocol published and the trial currently underway [108]. It is noteworthy that all of these clinical trials have used chimeric brain models as an integral component of their pre-clinical assessments, underscoring the pivotal role of these chimeric models in the rigorous evaluation of safety parameters and the transition to clinical application.

Improving the Chimerism of Human Cells in the Human–Animal Brain Chimeras

After xenograft into the neonatal or adult brain, human neural and immune cells migrate from the injection site to disseminated CNS regions, where they structurally and functionally integrate into the host brain. To achieve more effective disease modeling and cell therapy outcomes, several factors need to be considered to improve the chimerism: (1) Cell source quality and developmental stage play a critical role. Although hPSC-derived progenitor cells (NPCs [17, 40], GPCs [27], PMPs [25]), and the differentiated cell types (neurons [7], astrocytes [56], OPC [62], and microglia [25, 85]) are reported to generate chimeras, the progenitor cells allow extensive cell expansion *in vivo* and usually lead to a high degree of chimerism. As such, transplanting NPCs may result in more human neurons in chimeric brains compared with transplanting neurons directly [7, 40]; (2) The injection site is crucial to chimeric success. Multiple injection sites

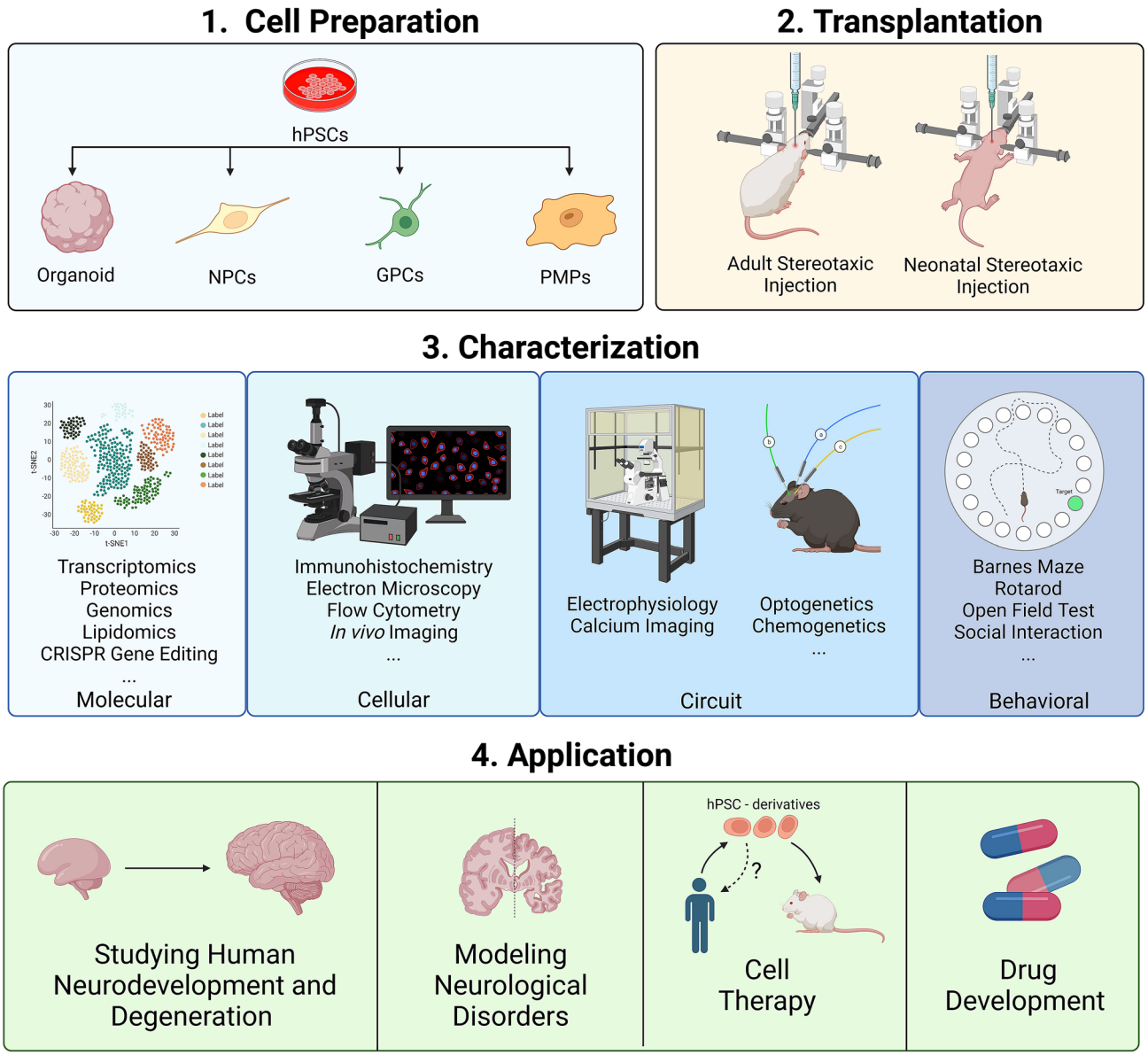

Fig. 4 Summary of cell preparation, transplantation, characterization, and application of human-animal brain chimeras. (1) Differentiation of hPSCs into glial progenitor cells, neural progenitor cells, primitive macrophage progenitors, and organoids. (2) Stereotaxic injection into neonatal or adult mice. (3) Characterization of the human-animal brain chimeras at molecular, cellular, circuit, and behavioral levels. (4) Various applications of chimeric models. The figure was generated by BioRender.

have been reported, including the lateral ventricles [7, 38, 109], frontal cortex [17], hippocampus [110], anlagen of the white matter [40], and the corpus callosum [27, 40, 66, 111] (Table 1). After transplantation, human cells can follow the intrinsic migration pattern, respond to developmental cues, unitize the established migratory pathways in the developing brain, and migrate to specific regions [28]. Selecting the appropriate injection site that mimics the human neurodevelopmental trajectory and migration patterns can promote high chimerism. For example, to generate interneuron chimeras, we previously transplanted hPSC-derived interneuron progenitors into the wall of the lateral ventricle, and this led to a wide distribution of interneurons in the cortex, mimicking the migration seen in the human infant brain [40]. In addition, to achieve extensive repopulation of the entire brain by donor-derived human cells, implantation of human cells into multiple sites is possible [27, 66, 85, 111]; (3) The injection time is also important. Generally, younger mice provide a more conducive environment for donor cell migration and yield better chimerism. Transplanting human cells into neonatal mice or shortly after birth is advantageous for generating high chimerism. However, when testing

cell therapy in adult mice, delivering human cells to specific brain regions is a different scenario; (4) The animal strain is also critical. To avoid rejection of human donor cells, multiple immunodeficient animals have been used, such as Rag1-/-, Rag2-/-, and NOD-SCID rodents (Table 1). Depending on the type of human-mouse brain chimera to be generated, the proper mouse strain needs to be selected. For instance, even when transplanting the same GPCs into immunodeficient mice, the GPCs preferentially differentiate into astrocytes or oligodendrocytes in different strains [12, 27]. For example, shiverer X rag2-/- immunodeficient mice have a mutation in MBP that causes a lack of functional oligodendrocytes in the host brain, allowing GPCs to differentiate into oligodendrocytes as the predominant option [27]. Thus, considering factors such as the quality and developmental stage of the cell source, injection site and time, and animal strain would be helpful in improving chimerism.

Improving the Integration and Maturation of Human Cells in Human–Animal Brain Chimeras

Previous studies demonstrated that human neural and immune cells (such as microglia) mature and functionally integrate into the host brain, and this might be determined by the intrinsic properties of the engrafted human cells and the host environment [7, 110]. Compared with neurodevelopment in animals, human neurodevelopment exhibits a prolonged timeline, consistently reported in hPSC-derived human neurons in chimeras [7]. For instance, while mouse neurons transplanted into the mouse cortex mature in only a few weeks, human neurons take ~11 months to reach the physiological maturation of adult human cortical neurons [7]. On the other hand, the transcriptomic profile of human microglia at 6 months post-transplantation in the host brain closely resembles that of adult human microglia [110], suggesting that the host brain environment accelerates the maturation of donor human cells. However, further studies are needed to determine if the maturation of human neural or immune cells can reach aging status.

To improve the maturation of human cells, transplanting donor cells with adult human cell features may be helpful. For example, the neurons (miR neurons) directly reprogrammed from adult fibroblasts by microRNA maintain adult neuron characteristics, including 4R tau expression [112]. Transplanting miR neurons might be valuable for studying neurodegeneration and neurodegenerative disorders, such as Alzheimer's disease. In addition, transplanting human cells into rats or non-human primates with longer life spans would enable the long-term survival and maturation of human donor cells.

Moreover, human neurons in the chimeras, whether transplanted as single-cell transplantation or organoid transplantation, can functionally integrate into host neural circuits [7, 24, 113]. Neonatal transplantation, occurring during the ongoing innervation and maturation of host neuronal circuits, provides an ideal environment for this integration. Previous studies have substantiated the presence of fully established synaptic connections between host cells and xenografted cells through various methodologies, encompassing morphological assessment, electrophysiology, and transsynaptic rabies experiments [43, 45]. For instance, human neurons transplanted at the neonatal stage can respond to visual stimuli, and optogenetic stimulation of human neurons can influence the behavior of the host animal [7].

The development of cell therapy, which requires the integration of transplanted cells into host neural circuits to establish functional synaptic connections [114], often takes place in adult animals with pre-existing mature neural circuits. However, due to factors such as differences in maturation tempo and anatomical structure between humans and other mammals, the proper integration of human neurons into host neural circuits, as found in the human brain, warrants further investigation. Encouraging data from previous studies suggested engrafting the proper cell type into appropriate brain regions is critical to reestablish the neural circuit in the host brain. For example, Xiong et al. demonstrated that transplantation of hESC-derived midbrain dopamine neurons, but not cortical glutamate neurons, into the midbrain of Parkinson's disease mice repair the nigro-striatal circuit anatomically and functionally [45].

Besides human neurons, there is ongoing research into the integration of glial and immune cells. Recent studies indicate that in adult animals, transplanted hGPCs can entirely replace disease-associated astrocytes [54]. In cases of demyelination or SCI in mice, transplanted OPCs have demonstrated the capacity to remyelinate the host CNS [64, 65]. Regarding microglia, neonatal transplantation can lead to chimeric brains with a substantial population of human microglia. However, for adult transplantation, it is necessary to deplete host microglia before implanting human microglia to achieve significant chimerism. Thus, optimization of the cell sources and injection method is critical for the effective integration of human cells into the host CNS.

Notably, characterization in multiple studies consistently confirms the retention of human characteristics by implanted cells, including the prolonged maturation period of human neurons. In addition, human neural cells maintain their heterogeneity within chimeric brains. For instance, human microglia display region-dependent morphologies and phenotypes [25, 86], while human astrocytes exhibit diverse morphologies, including the unique presence of interlaminar astrocytes, a feature exclusive to the human brain [12].

Developing Human-Animal Brain Chimeras with Human Innate and Adaptive Immune Cells

Another aspect for improving current human-animal brain chimeras is to incorporate adaptive immune cells, such as B cells and T cells, considering the increasing evidence suggesting their critical roles in brain development and neurological disorders [115, 116]. To avoid rejection and allow long-term survival of donor human cells, current human-animal brain chimeras mainly use immunodeficient mice (Table 1), which lack a complete peripheral adaptive immune system. Notably, significant species differences also exist between human and animal adaptive immune cells. Thus, generating human-animal chimeras that incorporate human neural, innate, and adaptive cells will enable the investigation of human adaptive cells' function and the interactions among human neural, innate immune, and adaptive immune cells in health and disease.

Previous studies have demonstrated that co-transplantation of neural cells and adaptive immune cells is a feasible approach that may lead to better therapeutic outcomes. In a recent study, Park et al. found that co-transplantation of regulatory T cells with hiPSC-derived midbrain DA neurons from the same patient improved the survival of midbrain DA neurons (mDANs) by resolving a needle trauma during surgery [117]. This addressed the critical issue of poor survival of hPSC-derived mDANs in transplantation-based cell therapy for Parkinson's disease. Moreover, previous studies have successfully generated hematopoietic stem progenitor cells (HSPCs), human T cells, and B cells from hiPSCs [118–122]. In the future, simultaneous transplantation of neural derivatives (such as neuronal, astroglial, and oligodendroglial cells), microglia, and HSPCs (or T cells and B cells) from the same disease-specific human iPSCs will potentially lead to a new version of the human-mouse chimera, incorporating both human neural cells and innate and adaptive immune cells derived from the same human donor.

In conclusion, human-animal chimeric brain models provide valuable tools for investigating the pathophysiology of human neural cells *in vivo* within the intact brain. Meanwhile, *in vitro,* hPSC-based 2D and 3D models are useful for high throughput screening, and animal models are powerful for studying organ systems and interactions. Therefore, integrating complementary hPSC-based *in vitro* 2D and 3D models, human-animal chimeric brain models, and traditional animal models holds great promise in enhancing our understanding of human brain development, unraveling the underlying mechanisms, and developing cell therapy for neurological disorders.

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Conflict of interest The authors declare that they have no conflict of interest.

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