PHILOSOPHICAL TRANSACTIONS B

rstb.royalsocietypublishing.org

Review

Cite this article: McComish SF, Caldwell MA. 2018 Generation of defined neural populations from pluripotent stem cells. *Phil. Trans. R. Soc. B* **373**: 20170214.
http://dx.doi.org/10.1098/rstb.2017.0214

Accepted: 6 March 2018

One contribution of 18 to a theme issue 'Designer human tissue: coming to a lab near you'.

Subject Areas:
cellular biology, developmental biology, neuroscience

Keywords:
iPSC, disease modelling, neurons, glia, neuralisation, neurodegeneration

Author for correspondence:
Maeve A. Caldwell
e-mail: maeve.caldwell@tcd.ie

Generation of defined neural populations from pluripotent stem cells

Sarah F. McComish and Maeve A. Caldwell

Department of Physiology, Trinity College Institute for Neuroscience, Trinity College Dublin, Dublin 2, Ireland

SFM, 0000-0003-0297-6907; MAC, 0000-0001-7442-711X

Effective and efficient generation of human neural stem cells and subsequently functional neural populations from pluripotent stem cells has facilitated advancements in the study of human development and disease modelling. This review will discuss the established protocols for the generation of defined neural populations including regionalized neurons and astrocytes, oligodendrocytes and microglia. Early protocols were established in embryonic stem cells (ESC) but the discovery of induced pluripotent stem cells (iPSC) in 2006 provided a new platform for modelling human disorders of the central nervous system (CNS). The ability to produce patient- and disease-specific iPSC lines has created a new age of disease modelling. Human iPSC may be derived from adult somatic cells and subsequently patterned into numerous distinct cell types. The ability to derive defined and regionalized neural populations from iPSC provides a powerful *in vitro* model of CNS disorders.

This article is part of the theme issue 'Designer human tissue: coming to a lab near you'.

1. Introduction

Effective and efficient modelling has been a major focus of disease research since the birth of pathology and although countless breakthroughs have been made over the years it is still arguably the limiting factor for translating therapies from 'bench-to-bedside'. Approaches to central nervous system (CNS) research have used immortalized cell lines, e.g. HEK293 and SH5Y5Y, but these immortalized cell lines have intrinsic flaws as they are often derived from cancers and may not be from the tissue affected by the disease of interest [1]. Additionally, cell lines are by definition highly proliferative and therefore do not provide adequate models for slowly proliferating cells such as neurons and microglia [2]. Primary cultures of neurons, astrocytes and microglia isolated from rodents have also been used but these primary cells have issues of their own, as they are often difficult to maintain and expand in culture, particularly when isolated from an aged animal. The problems associated with these approaches highlight a need for human cells, which can be grown *in vitro* while behaving as they do *in vivo*. An alternative route to disease modelling has used model organisms. Rodents are the traditional model organism of choice for CNS research, where the animals are genetically modified in order to induce the disease of interest; for example, the APP/PS1 mouse model of Alzheimer's disease (AD) over expresses amyloid precursor protein, resulting in the characteristic amyloid pathology and cognitive impairment associated with AD [3]. The mouse is the most prevalent animal model used in research today, despite the fact that there are many differences between mice and humans both genetically and phenotypically, e.g. heart size and resting heart rate [1]. In terms of evolution the rat is 4–5 million years closer to humans compared to mice [4] and is commonly used as the main model organism in Parkinson's disease (PD) research. Although these model organisms have contributed to elucidating key pathological mechanisms, the use of non-human models of a human disease is inherently flawed given that rodents do not naturally develop many disorders of the CNS [5]. This has resulted in difficulties translating therapies developed in rodent models into feasible therapies as therapeutics viable in one species may be detrimental in the other [6,7].

THE ROYAL SOCIETY PUBLISHING

A human model in theory would create a more realistic representation of human disease. Human pluripotent stem cells (PSC) present a means by which a human model can be created. Neuronal and astrocyte cultures may be differentiated from human PSC to provide a 'disease in a dish' model of CNS disorders.

Study of neural disorders has previously been hindered by the lack of live human neural cells, and while cell lines and model organisms have their uses and have contributed to much of what we know about neurodegeneration to date, there is a need for a human model in order to more effectively and efficiently translate this from 'bench-to-bedside'. Therefore, research turned towards generating a human model of human disease with initial studies using embryonic stem cells (ESC). Further to this, the development of PSC technology has allowed researchers to generate defined neural populations *in vitro*, and has subsequently resulted in a number of breakthroughs in studying neural development and neurological diseases, e.g. elucidating the role of bone morphogenic protein (BMP), Wnt, retinoic acid (RA) and sonic hedgehog (SHH) in embryonic rostro-caudal dorso-ventral patterning [8], as well as the ability to produce patient-specific lines for disease modelling. Induced pluripotent stem cell (iPSC) generation was first demonstrated in mouse dermal fibroblasts in the laboratory of Shinya Yamanaka [9] and subsequently in human dermal fibroblasts [10,11]. The ability to produce regionally specified neural cells allows research to focus on the cell populations most vulnerable in the disease of interest, e.g. basal forebrain cholinergic neurons in AD, and therefore cater therapies to a specific cell type. These defined neural phenotypes include: (i) basal forebrain cholinergic neurons—relevant to AD research, (ii) cortical projection neurons—relevant to AD research, (iii) cerebellar neurons—affected in multiple sclerosis (MS) and hereditary ataxia [12], (iv) midbrain dopaminergic neurons in order to study PD, and (v) motor neurons are useful for research into motor diseases such as amyotrophic lateral sclerosis (ALS) [13].

2. Pluripotent stem cells

PSC have the potential to differentiate into any cell type in the body. These cells have the ability to expand indefinitely and may be prompted with the appropriate factors to differentiate into a cell type of interest. Currently there are two sources of human PSC, firstly human ESC derived from the blastocyst and secondly, human iPSC derived from adult somatic cells that are genetically manipulated into a pluripotent state.

ESC are derived from the inner cell mass of the blastocyst. They have many uses in research including investigation of disease mechanisms, drug screening and regenerative medicine. However, obtaining human embryonic tissue is difficult, and there are many ethical controversies surrounding the use of human ESC as they require destruction of viable embryos; additionally generation of disease- or patient-specific ESC is limited. The introduction of iPSC has provided an alternative approach that bypasses some of the limitations of ESC [14,15]. iPSC have much of the same characteristics and differentiating abilities of ESC without the ethical issues associated with the use of human embryos; furthermore, autologous cells have a reduced risk of rejection by the host following implantation [16,17]. Early protocols used mouse and human ESC to establish differentiating techniques (for review see [8]); however, iPSC are more commonly used today, and as such this review will focus on neural patterning of human iPSC.

3. Induced pluripotent stem cells

Human iPSC are adult somatic cells that have been reprogrammed into a primordial state much like ESC, and thus in theory can give rise to any cell type of the body. They were first derived in 2006 in the laboratory of Shinya Yamanaka from mouse skin cells [9] and the first human iPSC were derived in 2007 [10,11]. This pioneering research has revolutionized disease modelling and is considered such a breakthrough that Shinya Yamanaka was awarded the Nobel Prize in Medicine in 2012. The real beauty of these cells is that they may be derived from any willing donor and a corresponding iPSC line containing the donor's genetic fingerprint can be produced and subsequently differentiated into the desired cell type, therefore disease- and patient-specific cell lines may be produced to model diseases arising from a genetic mutation. Human iPSC derived from tissue collected from AD patients will naturally contain any mutations that led to the development of AD in that individual, and thus bypass the need for transgenically inducing the disease.

Takahashi and colleagues showed that iPSC generated from mouse resembled ESC with regards to morphology, gene expression, proliferation and formation of teratomas—and can give rise to adult chimeras capable of germline transmission when transplanted into blastocysts [10]. With appropriate stimulation iPSC can give rise to multiple cell types such as neurons, astrocytes, cardiomyocytes, pancreatic cells and liver cells [18,19], making them extremely versatile in terms of modelling disease. As iPSC are derived from tissues from consenting adults this bypasses the ethical limitations of ESC associated with the destruction of embryos and allows for the generation of disease- and patient-specific cell lines. However, genetic manipulation of human tissue is closely regulated and ethical approval for harvesting of human tissue is stringent—particularly gaining informed consent from dementia patients [20,21]. iPSC technology has shown many applications in the fields of drug development, disease modelling, organ synthesis and tissue repair. These cells have the advantages of a normal karyotype and continuous self-renewal, which allows them to survive in culture indefinitely. Therefore, the introduction of iPSC technology has helped overcome many limitations associated with the use of animal models and ESC, and in so doing is helping to bridge the 'gap' that exists between the laboratory and the clinic.

A number of steps are involved in the generation of iPSC prior to differentiation, namely sample collection, parent cell isolation and expansion, transfection, culture, expansion and characterization. iPSC may be derived from a number of sources including skin fibroblasts, mesenchymal stem cells from bone marrow, hair follicles, mononuclear cells from peripheral blood, or even exfoliated renal epithelial cells from urinary sediments. Skin fibroblasts are the traditional source of iPSC, as the method of isolation is minimally invasive [22]. Skin fibroblasts are isolated by taking a dermal punch from the forearm of the participant; this skin section is then cultured to encourage growth of fibroblasts. Early protocols forced somatic cells into a pluripotent state by means of retroviral transduction, whereby embryonic transcription factors (*OCT3/4*, *c-MYC*, *SOX2* and *KLF4*) are introduced into the adult cells. Changes to the Yamanaka group's original protocol have introduced the use of different vectors and reprogramming genes. The latest protocols use non-integrating episomal plasmid vectors to introduce the embryonic gene cocktail and the

non-transforming form of *c-MYC*, *L-MYC* [23]; *L-MYC* is now used routinely, thereby reducing the concern that these cells may form teratomas or become cancerous if implanted. Once transfected, the cells are cultured until iPSC colonies begin to appear in culture; these colonies are then isolated and expanded to produce a pure iPSC culture, free from fibroblast contamination. iPSC then undergo a series of characterization tests to prove pluripotency. Pluripotency characterization tests include the following:

(1) Morphology and gene expression are examined and compared to ESC
(2) Silencing of the retroviral transgenes (if used) should occur after approximately four passages
(3) iPSC form embryoid bodies with markers for all three germ layers demonstrated by immunostaining and RT-PCR
(4) Injection of undifferentiated iPSC colonies into immunodeficient mice results in the formation of teratomas [24]

4. Neural differentiation

Over the past decade, the introduction of technologies capable of reprogramming human somatic cells into human iPSC has provided a novel approach to studying neurodegenerative diseases *in vitro*, and resulted in a greater understanding of the pathophysiology of numerous neurological diseases. Since the protocol was first published, many new protocols have been established and it is now possible to generate a variety of defined neuronal phenotypes from these iPSC, for example dopaminergic and cortical neurons and astrocyte populations [5].

PSC can be differentiated into defined neural subtypes by modulating exogenous levels of signalling molecules present in the brain at significant developmental time points such as BMP, Wnt, sonic hedgehog SHH and fibroblast growth factor (FGF) [8]. By mimicking cues that guide neural development in the embryo, it is possible to generate regional specific neural progenitors that can then be matured into neurons and glial cells in order to model human development and disorders of the CNS in a way that was unattainable previously. Human PSC can be differentiated using a number of protocols, e.g. monolayer, on plastic and in defined chemical conditions. There is some debate within the community as to which protocol is more effective and reproducible, with both neurosphere and adherent monolayer cultures widely used [25]. While neurospheres recapitulate the environment within the embryo, i.e. a 3D bundle of cells [26,27], monolayers can often be easier to differentiate and result in a more homogeneous population [5]. Producing defined neuronal populations allows the focus to be placed on the neuronal subtypes affected in neurological disorders, e.g. cholinergic and cortical neurons may be used in AD-focused studies [27,28], while midbrain dopaminergic neuronal populations are useful when studying PD [29] and spinal cord neurons and astrocytes are required to study spinal cord injury [30,31].

5. Differentiation of neuronal subtypes

(a) Basal forebrain cholinergic neurons

The basal forebrain is considered the major source of acetylcholine within the CNS and the main source of cholinergic input to the cortex [32]. Basal forebrain cholinergic neurons (BFCN) are the earliest population of neurons to be affected by tau pathology in AD, resulting in loss of cholinergic input to the cortex and subsequently cognitive decline [28,32]. Cognitive impairment in AD has been linked to a loss of nicotinic acetylcholine receptors (nAChR) [26], and as such dysfunction of the cholinergic system. Therefore, these neuronal subtypes provide an extremely useful model to analyse the efficacy of drugs designed to combat neuronal loss in AD. iPSC derived from AD patients may be differentiated into BFCN and used to elucidate why this cell population is so vulnerable to AD.

Factors present in the forebrain that result in BFCN development include retinoic acid (RA), SHH, FGF8 and BMP9. These factors must be present in the right combination, at the right time and at the right concentrations in order to differentiate BFCN. The Kessler lab elucidated a mechanism of BFCN differentiation in 2011, whereby patterning factors (RA, SHH, FGF8, BMP9) result in the transcription of the genes *Lhx8* and *Gbx1*, subsequently leading to the production of a population of cells positive for the basal forebrain markers ChAT and p75 (table 1). Removal of BMP9, or addition at incorrect time points and knockdown of *Lhx8* or *Gbx1* resulted in the generation of a neuronal population positive for the neuronal marker MAP2 but none of the BFCN-specific markers [32]. Functional cholinergic neurons will provide a source for the screening of drugs targeting the cholinergic system, or a useful model for disease involving dysfunction of the cholinergic system.

(b) Cortical

The primate cortex is different from that of the rodent in the following ways: it is significantly larger relative to the rest of the CNS, it is more complex and has a more diverse neuronal cell population [33]. Therefore, iPSC have the potential to overcome the challenges linked to the production of animal models of disease of the cortex, including AD and schizophrenia. Some studies have found that cortical neuron fate is determined *in vitro* prior to implantation [41], while other studies have found that implanted cortical neurons develop dendritic and axonal connections applicable to the transplant site indicating that their fate is influenced by the environment [42]. Dual-SMAD inhibition has successfully been used to derive cortical neurons from human iPSC [43] (table 1).

(c) Midbrain dopaminergic neurons

Dopamine neurons of the substantia nigra pars compacta are the most vulnerable cells in PD. Significant loss of dopamine neurons results in the characteristic motor symptoms of PD due to a loss of dopamine in the striatum [44]. Given that idiopathic and genetic PD result from multiple genetic mutations, iPSC are proving a powerful model following derivation of midbrain dopaminergic neurons to study the intricacies of this debilitating disorder (see table 1 for derivation protocols).

(d) Spinal motoneurons

Spinal motoneurons are the key effector cells of motor function, relaying signals generated in the motor cortices of the brain to the muscles. The loss of motor neurons has been associated with a number of movement disorders, including ALS [39]. The generation of protocols to derive motoneurons from human iPSC has not only provided a model for disease

Table 1. PSC-derived regional specific neurons. Retinoic acid (RA), fibroblast growth factor (FGF) 8/2, human induced pluripotent stem cells (hiPSC), sonic hedgehog (SHH), human pluripotent stem cells (hPSC), basic fibroblast growth factor (bFGF), epidermal growth factor (EGF), nerve growth factor (NGF), fluorescence-activated cell sorting (FACS), choline acetyltransferase (ChAT), microtubule-associated protein 2 (MAP2), vesicular acetylcholine transporter (VAChT), insulin gene enhancer protein (ISL1), embryoid body (EB), bone morphogenic protein 9 (BMP9), neural progenitor cell (NPC), brain-derived growth factor (BDNF), neurotrophin (NT)-3/4, ciliary neurotrophic factor (CNTF), glial cell-derived neurotrophic factor (GDNF), ascorbic acid (AA), transforming growth factor beta (TGFβ), dibutyryl cyclic-AMP (db-cAMP), tyrosine hydroxylase (TH), neuron-specific Class III β-tubulin (TuJ1), motoneuron (MN), insulin-like growth factor 1 (IGF1).

brain region	patterning factors	neurotrophic factors	initial differentiation	method of differentiation	time required for mature neurons/yield	markers	references
basal forebrain cholinergic neurons	RA FGF8 SHH	bFGF EGF NGF	hiPSC neurosphere	Treatment with bFGF, RA, EGF, FGF8 and SHH. Transfected with Lhx8/Gbx1-IRES-GFP, FACS sorted.	**∼24 d** ∼60% ChAT^{+}/MAP2^{+}	Lhx8 Gbx1 VAChT Nkx2.1	[28]
	SB431542	FGF2 EGF	hPSC embryoid body	EB-based, non-adherent differentiation.	**∼80 d** >90% ChAT^{+}	ChAT VAChT ISL1 P75^{NTR} *Nkx2.1* *Lhx8*	[27]
	RA SHH FGF8 BMP9	bFGF	ESC neurosphere	Cells induced with bFGF, RA, FGF8 and SHH. BFCN were derived from NPC by addition of BMP9 or nucleofection with *Lhx8*, *Gbx1*.	**35–40 d** 94% ChAT^{+}/MAP^{+}	ChAT P75 *Lhx8* *Gbx1*	[32]
	NGF BDNF NT-3 CNTF	EGF bFGF BDNF CNTF NT-4 NGF	hESC neurosphere	1. Suspension culture: neurospheres expanded in bFGF and EGF. 2. Adherent culture: neurospheres dissociated, plated and treated with BDNF, CNTF, NT-4 and NGF.	**>70 d** 76 ± 1.8% *Nkx2.1* >69% ChAT	P75^{NTR} *Gsh2* *Dlx1/2* *Gbx2* *Nkx2.1* *Lhx6/8* ChAT Nkx2.1 Islet-1	[26]

(*Continued.*)

Table 1. (*Continued.*)

brain region	patterning factors	neurotrophic factors	initial differentiation	method of differentiation	time required for mature neurons/yield	markers	references
cortical projection neurons	dorsomorphin/noggin SB431542	N2 B27 FGF2	hESC hiPSC	SMAD inhibition (noggin/dorsomorphin and SB431542; 12 days). NPC expanded with N2 and B27. FGF2 added to neural rosettes. Neurons maintained for up to 80 days. Early projection neurons ~2–3 weeks after initiation of induction. Layer 2 neurons ~90 d	**>80 d** >90% $Pax6^+$ 45–50 d synapses appear 2–3 months neural networks appear	deep-layer: Tbr1 CTIP2 upper-layer: Satb2 Cux1 Brn2	[33]
midbrain dopaminergic neurons	SB431542 noggin SHH-C24II CHIR99021	FGF8b BDNF AA GDNF DAPT db-cAMP	hPSC	Dual SMAD inhibition (SB431542, noggin), SHH, CHIR and FGF8b. From day 16+ NPC may be matured by plating and addition of BDNF, AA, GDNF, db-cAMP and DAPT.	**~16 d for NPC** **>40 d for mature neurons** ~80% $LMX1^+/FOXA2^+$ ~95% $LMX1^+/OTX2^+$	TH LMX1 FOXA2 EN1 MAP2	[34,35]
	noggin SB431542 dorsomorphin SHH C24II Wnt1 Dkk1 blocking antibody	FGF2 BDNF GDNF AA FGF8 TGFβ3 db-cAMP	hiPSC monolayer protocol	SB431542, dorsomorphin, SHH C24II, Wnt1 and Dkk1 blocking antibody. NPC maintained in SHH, BDNF, AA and FGF8, and matured in BDNF, AA, GDNF, TGFβ3 and db-cAMP.	**>25 d** ~30% $TH^+/TuJ1^+$	α-synuclein TH LMX1B *LMX1A* *NURR1* *TH* *DAT*	[29]
	FGF8 SHH C24II LDN193189 SB431542 CHIR99021 purmorphamine	BDNF GDNF TGFβ3 AA db-cAMP DAPT	hESC monolayer	Cells induced with FGF8, SHH C24II, LDN193189, SB431542, CHIR99021 and purmorphamine, Expanded in media with CHIR, BDNF, GDNF, TGFβ3, AA, cAMP and DAPT.	**~60 d** >60% TH^+	TH	[36]

(*Continued.*)

Table 1. (*Continued.*)

brain region	patterning factors	neurotrophic factors	initial differentiation	method of differentiation	time required for mature neurons/yield	markers	references
striatal neurons	SB431542 LDN193189 dorsomorphin SHH cyclopamine activin A	BDNF GDNF	hiPSC monolayer	Cells induced with SB, LDN, dorsomorphin, SHH, cyclopamine for 10 d. Activin A added at day 9. Neurons matured in BDNF, GDNF.	**∼40 d** >50% CTIP2^{+} >50% GSX2^{+} >30% DARPP32^{+}	CTIP2 GSX2 NKX2.1 MAP2 DARPP32	[37,38]
	Dorsomorphin/noggin SB431542 SHHC-25II DKK1	BDNF	hESC hiPSC Adherent monolayer	Dual SMAD inhibition with dorsomorphin/noggin and SB431542. Striatal differentiation with SHHC-25II and DKK1. Neurons matured in media with B27 and BDNF.	**> 45 d** 80% β-tubulin III^{+} 58% FOXG1^{+} 67% GSX2^{+} ∼ 30% MAP2ab^{+}/CTIP2^{+}	FOXG1 GSX2 LIX1 ZNF503 CXCR7 CTIP2 DARPP-32 CALB1 GAD65/67 GABA ARPP21	[38]
spinal motor neurons	SB431542 dorsomorphin N-acetylcysteine RA	purmorphamine bFGF BDNF GDNF forskolin	hiPSC hESC	Dual SMAD inhibition and RA. Cells expanded in purmorphamine and FGF. Neurons matured in BDNF, GDNF, and forskolin.	**∼70 d** >80% nestin^{+} Sox1^{+} ∼50% HB9^{+}	nestin Sox1 HB9 SMI-32	[24]
	SHH RA noggin	AA BDNF	hESC neural rosette	Cells induced MS5 stromal cells with SHH, RA, noggin. Cells maintained in media with AA, BDNF and GDNF.	**>60 d** 20% HB9^{+}	Olig2 HB9	[39]
	RA SHH cAMP	FGF2 BDNF GDNF IGF1	hESC neural rosette to adherent monolayer	Cells induced with RA, SHH and cAMP. Expanded with BDNF, GDNF and IGF1.	**∼50 d** ∼21% HB9^{+}	HB9 HoxC8 ChAT VAChT	[40]

Table 2. PSC-derived astrocytes. Platelet-derived growth factor (PDGF), human leukaemia inhibitory factor (hLIF), aldehyde dehydrogenase 1 family member L1 (ALDH1L1), glial fibrillary protein (GFAP), excitatory amino acid transporter 1 (EAAT1), glutamine synthetase (GS), astrocyte precursor cell (APC), nuclear factor 1A (NF1A), creatine transporter (CT1), aquaporin (AQ)4.

brain region	patterning factors	astrogliogenic factors	initial differentiation	method of differentiation	time required for mature astrocytes/yield	markers	references
n/a	noggin PDGF-AA	FGF2 EGF hLIF	hiPSC	SMAD inhibition followed by JAK/STAT induction.	**>50 d** $>$80% CD44$^+$ $\sim$90% S100β^+ $\sim$50% ALDH1L1$^+$	ALDH1L1 CD44 S100 β GFAP	[52]
cortical	FGF2 CNTF BMP2	EGF insulin	hiPSC-derived NPC Monolayer	Astrocyte differentiation was induced with medium modified from Shaltouki *et al*. (FGF2, CNTF and BMP2) [53] and addition of EGF and insulin.	**$\sim$30 d** $>$95% GFAP$^+$	GFAP EAAT1 S100β GS	[54]
n/a	SB431542 dorsomorphin N-acetylcysteine RA purmorphamine bFGF BDNF GDNF forskolin	EGF LIF FGF CNTF	hiPSC neurospheres and monolayer	MN precursors generated as described [24]. Enriched for APC with EGF, LIF, expanded in EGF, FGF2. Terminally differentiation CNTF	**>100 d** 90.6 $\pm$ 0.7% GFAP$^+$ 91.9 $\pm$ 1.9% S100β^+	vimentin NF1A GFAP EAAT1 *S100β* *GFAP*	[55]
n/a	CT-1 JAG1 noggin SB431542	FGF2 EGF CNTF	ESC iPSC embryonic body protocol	EB dissociated, treated with FGF2, EGF, and CNTF. Once neural rosettes formed noggin and SB431542 used for terminal differentiation.	**$\sim$35 d** 55–70% GFAP$^+$	GFAP *S100* *GLT-1*	[56]

(*Continued*.)

Table 2. (*Continued.*)

brain region	patterning factors	astrogliogenic factors	initial differentiation	method of differentiation	time required for mature astrocytes/yield	markers	references
n/a	BMP4	FGF2	hESC	hESC-derived neurospheres plated as a monolayer.	**>60 d**	GFAP	[57]
	LIF	EGF		Expanded in FGF2 and EGF; 2 months. Cells treated with	95.7 ± 3.1% $GFAP^+$	S100β	
			neurosphere	BMP2, BMP4 and LIF for terminal differentiation.	79.4 ± 1.0% $AQP4^+$	AQP4	
					90.1 ± 2.0% $S100β^+$	EAAT1	
					89.5 ± 3.2% $EAAT1^+$		
n/a	FGF8	EGF	hPSC	Cells treated with FGF8, RA or SHH for rosette formation.	**>90 d**	GFAP	[58]
	RA	FGF2		Rosettes patterned with EGF, FGF2. Matured with CNTF.	$>90\%$ $S100β^+$	S100β	
	SHH				$>90\%$ $GFAP^+$	CD44	
						Nkx2.1	

affecting this cell population but also allowed further probing of development of the CNS.

6. Astrocytes

Astrocytes are the most abundant cell type in the mammalian brain, and their main function is to maintain a homeostatic environment that is optimal for neuronal health and function. They do this by maintaining the blood–brain barrier (BBB), ion balance, neurotransmitter turnover and releasing growth factors that aid synapse formation. Given that neurons only represent 10% of cells in the adult brain [45] protocols for the differentiation of astrocytes, which represent up to 40% of brain cells [5], from PSC have been developed in an attempt to give a more representative disease model. There is a growing body of literature to support a detrimental role for astrocytes in neurodegeneration [30,46–48], with a recent study by Liddelow and colleagues attempting to characterize activated astrocytes [46] much like previous characterization of activated macrophages and microglia [49]. It has been established that reactive astrocytes have a role to play in many CNS diseases (for astrocyte review see [5,50], for glial cell review see [51]); as such it is vital that this cell type be included when studying neurodegenerative diseases and other brain disorders in order to give a more rounded and accurate representation of the *in vivo* environment.

Astrocytes are most commonly grown from rodent brain samples and cultures are typically contaminated with other cell types, e.g. microglia, therefore deriving astrocytes from iPSC is a convenient method of obtaining disease-specific astrocyte cultures of high purity. Astrocytes are a heterogeneous cell population, and much like neurons have different subtypes related to location, morphology, etc. The ability to generate specific defined astroglial populations from iPSC allows for research to focus on the brain regions and specific cell types affected in neurological diseases. Due to regional differences it is important to characterize these astrocyte populations and use the appropriate region when modelling disease. Table 2 summarizes available protocols for the generation of astrocytes from iPSC.

7. Oligodendrocytes

Oligodendrocytes are the myelinating cells of the CNS and while their dysfunction has been implicated in many diseases including the demyelinating disorders MS and ALS, their development and maturation in humans have yet to be fully determined [59]. Like astrocytes, oligodendrocytes may be regenerative or deleterious in disease states, and as such iPSC-derived oligodendrocytes are providing a means by which their developmental process can be elucidated and their response to disease subsequently examined. According to Mertens and colleagues, sufficient myelination is only achieved following implantation of oligodendrocytes [60], therefore improvements to current differentiation protocols will prove useful for research on demyelinating disorders such as MS, ALS and Krabbes disease. While oligodendrocytes have been successfully generated from iPSC in numerous laboratories following a variety of protocols (table 3), there is still a need for regional specificity in order to more selectively produce *in vitro* models of oligodendrocyte dysfunction in disease.

Table 3. PSC-derived oligodendrocytes. Smoothened agonist (SAG), triiodothyronine (T3), oligodendrocyte (OL), galactocerebrosidase (GALC), myelin basic protein (MBP), cyclic nucleotide phosphodiesterase (CNP), myelin-associated glycoprotein (MAG), hepatocyte growth factor (HGF), oligodendrocyte precursor cell (OPC), chondroitin sulphate proteoglycan (NG2), neuroepithelial (NE).

brain region	patterning factors	trophic factors/ terminal differentiation factors	initial differentiation	method of differentiation	time required for mature glia/yield	markers	references
n/a	SB431542 dorsomorphin CHIR99021 purmorphamine AA SAG	PDGF NT3 IGF1 AA T3 db-cAMP	iPSC EB followed by monolayer	Patterning with SB431542, dorsomorphin, CHIR99021 purmorphamine, AA and SAG. NPC transfected with *SOX10*, *OLIG2*, *ASCL1*, *NKX2.2*, *NKX6.1*, *NKX6.2*, *MYT1* and *RFP*. Cells differentiated to OL with SAG, PDGF, NT3, IGF-1, AA and T3 matured by db-cAMP.	**∼45 d** 70% $O4^+$	OLIG2 O4 GALC MBP CNP MAG	[61]
n/a	N-acetyl cysteine activin inhibitor dorsomorphin RA purmorphamine	FGF2 PDGFα SAG IGF-1 T3 ITS	ALS patient derived-iPSC EB/neurosphere protocol	EB plus dual SMAD inhibition, RA and purmorphamine. Maintained in FGF2, PDGFα, SAG, IGF-1, T3 and 1 X ITS. Matured to oligodendrocytes by mitogens withdrawal.	**>120 d** >65% $O4^+$ >80% $O4^+$/MBP^+	OLIG2 O4 MBP	[59]
n/a	noggin FGF2 EGF PDGF-AA		iPSC EB protocol	EB plus noggin and FGF2. NPC treated with EGF to yield glial progenitors and subsequently with EGF and PDGF-AA to produce OPC.	**>60 d** >98% $O4^+$	O4	[62]
n/a	SB431542 LDN RA PDGF IGF-1 HGF NT3	insulin T3 biotin cAMP AA	iPSC adherent and suspension culture	Dual SMAD inhibition and RA, followed by SAG. Cells plated in monolayer in media with PDGF, IGF-1, HGF and NT3. Subsequently in glial medium to mature oligodendrocytes (insulin, T3, biotin, cAMP, AA).	**∼95 d** 43.6–70% $O4^+$ (for different patient iPSC lines)	OLIG2 O4 NKX2.2 SOX10 NG2 MBP	[63]
n/a	bFGF FGF2 RA purmorphamine	T3 NT3 IGF PDGF-AA	iPSC embryoid body suspension neuralisation protocol	Low-adherent culture flasks and bFGF until EB developed then RA was added to yield NPC. Purmorphamine, bFGF added to yield pre-OPC. Glia induction by PDGF-AA, IGF and NT3.	**>120 d** >70% $OLIG2^+$/ $NKX2.2^+$	OLIG2 PDGFRα NKX2.2 SOX10 MBP	[64]

(*Continued*.)

Table 3. (*Continued.*)

brain region	patterning factors	trophic factors/ terminal differentiation factors	initial differentiation	method of differentiation	time required for mature glia/yield	markers	references
n/a	RA	FGF2	hESC	RA and SHH pattern the NE to a ventral spinal fate and FGF2 is then added to produce pre-OPC. Then matured to OPC with T3, NT3, PDGF, cAMP, IGF-1, biotin.	**>90 d** ~80% PDGFRα$^+$	OLIG2	[65]
	SHH					Nkx2.2	
	T3					Sox10	
	NT3					PDGFRα	
	PDGF					NG2	
	cAMP					O4	
	IGF-1					MBP	
	biotin						

8. Microglia

Microglia are the resident immune cells of the CNS, dubbed the brain-resident macrophages, with important homeostatic functions including developmental synaptic pruning and clearance of necrotic and apoptotic cells and their debris. Increasing evidence supports a role of microglia in CNS disorders, notably neurodegenerative diseases such as AD and PD. Many genes that have been linked to these disorders are expressed by microglia [2] and as such they may be predisposed to induce an inflammatory environment or their capacity to defend brain tissue could be compromised. Due to the immune function associated with microglia it follows that they develop from a different lineage from that of other neural cells, which has resulted in difficulties deriving microglia from iPSC. Only recently has microglia ontogeny been more clearly elucidated and microglia derivation protocols are slowly emerging in the literature. It has been established that microglia derive from the myeloid lineage, they develop from the yolk sac at embryonic day 17 (E17) [66], migrate to the CNS from E31 onwards [67]—prior to BBB formation—and subsequently develop into specialized immune cells within the CNS environment.

Previously microglia-like cell lines have been used, although by nature such cell lines are highly proliferative and don't fully recapitulate microglia as they behave *in vivo* [2]. Primary microglia may be isolated from rodent brain and cultured *in vitro* although they tend to lose their unique identity once removed from the brain environment. In addition, primary human microglia are in limited supply and do not proliferate *in vitro*. Therefore, microglia generated from iPSC lines will prove extremely useful for further investigating the role of microglia in neurodegeneration. Early attempts to produce human microglia using peripheral blood monocytes were trialled by adding factors such as macrophage colony-stimulating factor (M-CSF), granulocyte-macrophage colony-stimulating factor (GM-CSF), nerve growth factor (NGF) and chemokine ligand 2 (CCL2) [68]. This type of approach has allowed further protocols for the generation of microglia from iPSC to begin to emerge. Unlike protocols for the derivation of neurons and astrocytes, these iPSC-derived microglia lack regionality, something that we hope will be rectified in the future. The currently available microglia derivation protocols are summarized in table 4.

9. Conclusion and future perspectives

The scarcity of human CNS cells and the difficulty in isolating them have long hindered research into neural development and disease, and the polygenic nature of neurological diseases has created difficulties producing transgenic models for research. The ability to produce neural cells from human PSC and subsequently iPSC was a major breakthrough for the field of neuroscience research. Although iPSC are not without their limitations, with high variability present across cell lines and individual clones, it is clear that human PSC provide great advantages and opportunities for research into neural disorders and development of novel therapies to treat such disorders. Given that iPSC-derived neural cells can be transplanted and successfully integrate *in vivo* [73], these cells have created new avenues for regenerative medicine that may prove successful in the future to help combat neurodegeneration. iPSC have the added benefit that they can be derived

Table 4. PSC-derived microglia. Vascular endothelial growth factor (VEGF), stem cell factor (SCF), macrophage colony-stimulating factor (M-CSF), ionized calcium binding adaptor molecule 1 (Iba1), lithium chloride (LiCl), thrombopoietin (TPO), haematopoietic progenitor cell (HPC), triggering receptor expressed on myeloid cells 2 (TREM2), granulocyte-macrophage colony-stimulating factor (GM-CSF), granulocyte colony-stimulating factor (G-CSF), colony-stimulating factor 1 (CSF1).

brain region	patterning factors	trophic factors/terminal differentiation factors	initial differentiation	method of differentiation	time required for mature glia/yield	markers	references
n/a	BMP4 VEGF SCF IL-3	M-CSF	iPSC-derived macrophages	iPSC differentiated into macrophage/microglia precursors then differentiated into microglia and co-cultured with iPSC-derived neurons.	**>40 d**	Iba1 *MERTK* *GPR34* *PROS1* *C1QA* *GAS6* *P2RY12*	[2]
n/a	FGF2 BMP4 activin-A LiCl VEGF TPO SCF IL-6 IL-3	M-CSF IL-34 TGFβ-1 CD200 CX3CL1	iPSC monolayer	HPC generated with FGF2, BMP4, activin-A and LiCl for 2 d, followed by FGF2, VEGF for 2 d. TPO, SCF, IL-6, IL-3 for 6 d. Cells sorted for CD43. $CD43^+$ were expanded in M-CSF, IL-34 and TGFβ-1, differentiated to microglia-like cells with CD200, CX3CL1.	**>35 d** >97.2% $P2RY12^+$/ $TREM2^+$	Iba1 P2RY12 TREM2	[69]
n/a	BMP4 bFGF SCF VEGFA IL-3 TPO M-CSF FLT3 GM-CSF	IL-34 GM-CSF	iPSC monolayer	Myeloid differentiation: BMP4, bFGF, SCF, VEGFA, IL-3, TPO, M-CSF, FLT3 and GM-CSF. FACS sorting isolated $CD14^+$/$CX3CR1^+$ microglia progenitors. Differentiation to microglia with IL-34, GM-CSF.	∼**60 d** 95.9% $CD11c^+$ 94.6% $CD11b^+$	Iba1 CD11c TMEM119 P2RY12 CD11b CX3CR1	[70]

(*Continued*.)

Table 4. (*Continued.*)

brain region	patterning factors	trophic factors/terminal differentiation factors	initial differentiation	method of differentiation	time required for mature glia/yield	markers	references
n/a	VEGF BMP4 SCF activin A FLT3 L IL-3 IL-6 G-CSF	IL-3 GM-CSF M-CSF	iPSC adherent monolayer	iPSC patterned with VEGF, BMP4, SCF activin A for 4 d. Media changed to SCF, FLT3 L, IL-3, IL-6, G-CSF and BMP4 until day 15 to produce HPC. Differentiation by co-culture with astrocytes with IL-3, GM-CSF, M-CSF for 1–2 weeks. $CD39^+$ cells isolated by FACS.	**∼30 d** 8.6% $CD39^+$ (isolated from astrocyte co-culture)	CD34 CD45 CD43 CD39 CD11b Iba1 HLA-DR *P2RY12* *GPR34* *MERTK* *C1QA* *PROS* *GAS6*	[71]
n/a	dorsomorphin bFGF	IL-34 CSF1	iPSC monolayer followed by EB suspension protocol	iPSC patterned with dorsomorphin, bFGF. NPC transferred media with IL-34, CSF1. EB regularly triturated and sheared for cells of interest, which were further expanded in IL-34 and CSF1 to derive mature microglia-like cells.	**∼70 d** 98% $Iba1^+$/$CD11b^+$ 98% $CD45^+$/$CD11b^+$	Iba1 PU.1 CD45 CD11b TMEM119 P2RY12 *PU.1*	[72]

from numerous types of adult somatic cells, and the resulting cell line will have the same genetic makeup as the donor. In this way, iPSC lines recapitulating the genome of patients of CNS disorders, e.g. *APOE* in AD, *LRRK2* in PD, etc. may be generated and provide a much more realistic and accurate model of the human condition. The ability to generate region specific neural populations carrying a disease phenotype is a significant step forward in disease modelling, understanding disease mechanisms and screening potential therapeutics. However, it is noteworthy that a significant number of challenges need to be overcome before these cells realize their true potential. It is clear that at present the ability to differentiate regional glial populations lags behind that of neuronal counterparts. In addition, the issue of ageing the neurons *in vitro* is not trivial; many protocols need 100 plus days to generate neurons that are akin to their fetal brain counterparts [74]. This is particularly important when modelling neurodegenerative disease where the biggest risk factor is ageing, which becomes significant beyond middle age [25]. Variability between iPSC clones also remains a major challenge (reviewed in [75]). Furthermore, time taken to optimize culture conditions can be costly and lengthy and things that may sound simple, e.g. plastic ware, brands of reagents used, can have a profound effect on cellular differentiation. Laboratory to laboratory variability of neural cultures may have an effect down the line when trying to translate potential drug candidates into the clinic. This variability is something that needs to be addressed and more tightly regulated in the drug discovery industry, and especially for cell replacement therapy. This necessity is slowly being realized in the industry with the introduction of 'clinical-grade' ESC and iPSC [76–78].

In summary, despite these limitations, iPSC will continue to make a valuable contribution to our efforts in finding novel treatments for neurodegenerative diseases; their true potential is only beginning to emerge.

Data accessibility. This article has no additional data.
Competing interests. We declare we have no competing interests.
Funding. We received no funding for this study.

References

1. Avior Y, Sagi I, Benvenisty N. 2016 Pluripotent stem cells in disease modelling and drug discovery. *Nat. Rev. Mol. Cell Biol.* **17**, 170–182. (doi:10.1038/nrm.2015.27)
2. Haenseler W *et al.* 2017 A highly efficient human pluripotent stem cell microglia model displays a neuronal-co-culture-specific expression profile and inflammatory response. *Stem Cell Rep.* **8**, 1727–1742. (doi:10.1016/j.stemcr.2017.05.017)
3. Carrera I *et al.* 2013 Immunocytochemical characterization of Alzheimer disease hallmarks in APP/PS1 transgenic mice treated with a new anti-amyloid-β vaccine. *BioMed Res. Int.* **2013**, 709145. (doi:10.1155/2013/709145)
4. Cohen RM *et al.* 2013 A transgenic Alzheimer rat with plaques, tau pathology, behavioral impairment, oligomeric Aβ, and frank neuronal loss. *J. Neurosci.* **33**, 6245–6256. (doi:10.1523/JNEUROSCI.3672-12.2013)
5. Crompton LA, Cordero-Llana O, Caldwell MA. 2017 Astrocytes in a dish: using pluripotent stem cells to model neurodegenerative and neurodevelopmental disorders. *Brain Pathol.* **27**, 530–544. (doi:10.1111/bpa.12522)
6. National Academies of Sciences EaM, Health and Medicine D, Board on Health Sciences P, Forum on Neurosciences and Nervous System D. 2017 The National Academies Collection: Reports funded by National Institutes of Health. Therapeutic Development in the Absence of Predictive Animal Models of Nervous System Disorders: Proceedings of a Workshop. Washington (DC): National Academies Press (US). Copyright 2017 by the National Academy of Sciences. All rights reserved.
7. McGonigle P, Ruggeri B. 2014 Animal models of human disease: challenges in enabling translation. *Biochem. Pharmacol.* **87**, 162–171. (doi:10.1016/j.bcp.2013.08.006)
8. Petros TJ, Tyson JA, Anderson SA. 2011 Pluripotent stem cells for the study of CNS development. *Front. Mol. Neurosci.* **4**, 30. (doi:10.3389/fnmol.2011.00030)
9. Takahashi K, Yamanaka S. 2006 Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell* **126**, 663–676. (doi:10.1016/j.cell.2006.07.024)
10. Takahashi K, Tanabe K, Ohnuki M, Narita M, Ichisaka T, Tomoda K, Yamanaka S. 2007 Induction of pluripotent stem cells from adult human fibroblasts by defined factors. *Cell* **131**, 861–872. (doi:10.1016/j.cell.2007.11.019)
11. Yu J *et al.* 2007 Induced pluripotent stem cell lines derived from human somatic cells. *Science* **318**, 1917–1920. (doi:10.1126/science.1151526)
12. Mormina E, Petracca M, Bommarito G, Piaggio N, Cocozza S, Inglese M. 2017 Cerebellum and neurodegenerative diseases: beyond conventional magnetic resonance imaging. *World J. Radiol.* **9**, 371–388. (doi:10.4329/wjr.v9.i10.371)
13. Crippa V, Galbiati M, Boncoraglio A, Rusmini P, Onesto E, Giorgetti E, Cristofani R, Zito A, Poletti A. 2013 Motoneuronal and muscle-selective removal of ALS-related misfolded proteins. *Biochem. Soc. Trans.* **41**, 1598–1604. (doi:10.1042/BST20130118)
14. Meskus M, de Miguel Beriain I. 2013 Embryo-like features of induced pluripotent stem cells defy legal and ethical boundaries. *Croat. Med. J.* **54**, 589–591. (doi:10.3325/cmj.2013.54.589)
15. Zheng YL. 2016 Some ethical concerns about human induced pluripotent stem cells. *Sci. Eng. Ethics* **22**, 1277–1284. (doi:10.1007/s11948-015-9693-6)
16. Araki R *et al.* 2013 Negligible immunogenicity of terminally differentiated cells derived from induced pluripotent or embryonic stem cells. *Nature* **494**, 100–104. (doi:10.1038/nature11807)
17. Zhao T, Zhang ZN, Rong Z, Xu Y. 2011 Immunogenicity of induced pluripotent stem cells. *Nature* **474**, 212–215. (doi:10.1038/nature10135)
18. Sanal MG. 2011 Future of liver transplantation: non-human primates for patient-specific organs from induced pluripotent stem cells. *World J. Gastroenterol.* **17**, 3684–3690. (doi:10.3748/wjg.v17.i32.3684)
19. Lai MI, Wendy-Yeo WY, Ramasamy R, Nordin N, Rosli R, Veerakumarasivam A, Abdullah S. 2011 Advancements in reprogramming strategies for the generation of induced pluripotent stem cells. *J. Assist. Reprod. Genet.* **28**, 291–301. (doi:10.1007/s10815-011-9552-6)
20. Davis DS. 2017 Ethical issues in Alzheimer's disease research involving human subjects. *J. Med. Ethics* **43**, 852–856. (doi:10.1136/medethics-2016-103392)
21. West E, Stuckelberger A, Pautex S, Staaks J, Gysels M. 2017 Operationalising ethical challenges in dementia research—a systematic review of current evidence. *Age Ageing* **46**, 678–687. (doi:10.1093/ageing/afw250)
22. Zhang W, Jiao B, Zhou M, Zhou T, Shen L. 2016 Modeling Alzheimer's disease with induced pluripotent stem cells: current challenges and future concerns. *Stem Cells Int.* **2016**, 7828049.
23. Okita K *et al.* 2011 A more efficient method to generate integration-free human iPS cells. *Nat. Methods* **8**, 409–412. (doi:10.1038/nmeth.1591)
24. Bilican B *et al.* 2012 Mutant induced pluripotent stem cell lines recapitulate aspects of TDP-43 proteinopathies and reveal cell-specific vulnerability. *Proc. Natl Acad. Sci. USA* **109**, 5803–5808. (doi:10.1073/pnas.1202922109)
25. Arber C, Lovejoy C, Wray S. 2017 Stem cell models of Alzheimer's disease: progress and challenges. *Alzheimers Res. Ther.* **9**, 42. (doi:10.1186/s13195-017-0268-4)

26. Nilbratt M, Porras O, Marutle A, Hovatta O, Nordberg A. 2010 Neurotrophic factors promote cholinergic differentiation in human embryonic stem cell-derived neurons. *J. Cell. Mol. Med.* **14**(6B), 1476–1484. (doi:10.1111/j.1582-4934.2009.00916.x)
27. Crompton LA *et al.* 2013 Stepwise, non-adherent differentiation of human pluripotent stem cells to generate basal forebrain cholinergic neurons via hedgehog signaling. *Stem Cell Res.* **11**, 1206–1221. (doi:10.1016/j.scr.2013.08.002)
28. Duan L, Bhattacharyya BJ, Belmadani A, Pan L, Miller RJ, Kessler JA. 2014 Stem cell derived basal forebrain cholinergic neurons from Alzheimer's disease patients are more susceptible to cell death. *Mol. Neurodegener.* **9**, 3. (doi:10.1186/1750-1326-9-3)
29. Devine MJ *et al.* 2011 Parkinson's disease induced pluripotent stem cells with triplication of the α-synuclein locus. *Nat. Commun.* **2**, 440. (doi:10.1038/ncomms1453)
30. Roybon L *et al.* 2013 Human stem cell-derived spinal cord astrocytes with defined mature or reactive phenotypes. *Cell Rep.* **4**, 1035–1048. (doi:10.1016/j.celrep.2013.06.021)
31. Kawabata S *et al.* 2016 Grafted human iPS cell-derived oligodendrocyte precursor cells contribute to robust remyelination of demyelinated axons after spinal cord injury. *Stem Cell Rep.* **6**, 1–8. (doi:10.1016/j.stemcr.2015.11.013)
32. Bissonnette CJ, Lyass L, Bhattacharyya BJ, Belmadani A, Miller RJ, Kessler JA. 2011 The controlled generation of functional basal forebrain cholinergic neurons from human embryonic stem cells. *Stem Cells* **29**, 802–811. (doi:10.1002/stem.626)
33. Shi Y, Kirwan P, Livesey FJ. 2012 Directed differentiation of human pluripotent stem cells to cerebral cortex neurons and neural networks. *Nat. Protoc.* **7**, 1836–1846. (doi:10.1038/nprot.2012.116)
34. Nolbrant S, Heuer A, Parmar M, Kirkeby A. 2017 Generation of high-purity human ventral midbrain dopaminergic progenitors for *in vitro* maturation and intracerebral transplantation. *Nat. Protoc.* **12**, 1962–1979. (doi:10.1038/nprot.2017.078)
35. Kirkeby A, Grealish S, Wolf DA, Nelander J, Wood J, Lundblad M, Lindvall O, Parmar M. 2012 Generation of regionally specified neural progenitors and functional neurons from human embryonic stem cells under defined conditions. *Cell Rep.* **1**, 703–714. (doi:10.1016/j.celrep.2012.04.009)
36. Kriks S *et al.* 2011 Dopamine neurons derived from human ES cells efficiently engraft in animal models of Parkinson's disease. *Nature* **480**, 547–551. (doi:10.1038/nature10648)
37. Arber C *et al.* 2015 Activin A directs striatal projection neuron differentiation of human pluripotent stem cells. *Development* **142**, 1375–1386. (doi:10.1242/dev.117093)
38. Delli Carri A *et al.* 2013 Developmentally coordinated extrinsic signals drive human pluripotent stem cell differentiation toward authentic DARPP-32+ medium-sized spiny neurons. *Development* **140**, 301–312. (doi:10.1242/dev.084608)
39. Lee H, Shamy GA, Elkabetz Y, Schofield CM, Harrsion NL, Panagiotakos G, Socci ND, Tabar V, Studer L. 2007 Directed differentiation and transplantation of human embryonic stem cell-derived motoneurons. *Stem Cells* **25**, 1931–1939. (doi:10.1634/stemcells.2007-0097)
40. Li XJ, Du ZW, Zarnowska ED, Pankratz M, Hansen LO, Pearce RA, Zhang S-C. 2005 Specification of motoneurons from human embryonic stem cells. *Nat. Biotechnol.* **23**, 215–221. (doi:10.1038/nbt1063)
41. Gaspard N *et al.* 2008 An intrinsic mechanism of corticogenesis from embryonic stem cells. *Nature* **455**, 351–357. (doi:10.1038/nature07287)
42. Ideguchi M, Palmer TD, Recht LD, Weimann JM. 2010 Murine embryonic stem cell-derived pyramidal neurons integrate into the cerebral cortex and appropriately project axons to subcortical targets. *J. Neurosci.* **30**, 894–904. (doi:10.1523/JNEUROSCI.4318-09.2010)
43. Chambers SM, Fasano CA, Papapetrou EP, Tomishima M, Sadelain M, Studer L. 2009 Highly efficient neural conversion of human ES and iPS cells by dual inhibition of SMAD signaling. *Nat. Biotechnol.* **27**, 275–280. (doi:10.1038/nbt.1529)
44. Rinaldi F, Caldwell MA. 2013 Modeling astrocytic contribution toward neurodegeneration with pluripotent stem cells: focus on Alzheimer's and Parkinson's diseases. *Neuroreport* **24**, 1053–1057. (doi:10.1097/WNR.0000000000000064)
45. Herculano-Houzel S. 2014 The glia/neuron ratio: how it varies uniformly across brain structures and species and what that means for brain physiology and evolution. *Glia* **62**, 1377–1391. (doi:10.1002/glia.22683)
46. Liddelow SA *et al.* 2017 Neurotoxic reactive astrocytes are induced by activated microglia. *Nature* **541**, 481–487. (doi:10.1038/nature21029)
47. Osborn LM, Kamphuis W, Wadman WJ, Hol EM. 2016 Astrogliosis: an integral player in the pathogenesis of Alzheimer's disease. *Prog. Neurobiol.* **144**, 121–141. (doi:10.1016/j.pneurobio.2016.01.001)
48. Lobsiger CS, Cleveland DW. 2007 Glial cells as intrinsic components of non-cell-autonomous neurodegenerative disease. *Nat. Neurosci.* **10**, 1355–1360. (doi:10.1038/nn1988)
49. Kanazawa M, Ninomiya I, Hatakeyama M, Takahashi T, Shimohata T. 2017 Microglia and monocytes/macrophages polarization reveal novel therapeutic mechanism against stroke. *Int. J. Mol. Sci.* **18**, 2135. (doi:10.3390/ijms18102135)
50. Capani F, Quarracino C, Caccuri R, Sica RE. 2016 Astrocytes as the main players in primary degenerative disorders of the human central nervous system. *Front. Aging Neurosci.* **8**, 45. (doi:10.3389/fnagi.2016.00045)
51. Kaminsky N, Bihari O, Kanner S, Barzilai A. 2016 Connecting malfunctioning glial cells and brain degenerative disorders. *Genomics Proteomics Bioinformatics* **14**, 155–165. (doi:10.1016/j.gpb.2016.04.003)
52. Santos R *et al.* 2017 Differentiation of inflammation-responsive astrocytes from glial progenitors generated from human induced pluripotent stem cells. *Stem Cell Rep.* **8**, 1757–1769. (doi:10.1016/j.stemcr.2017.05.011)
53. Shaltouki A, Peng J, Liu Q, Rao MS, Zeng X. 2013 Efficient generation of astrocytes from human pluripotent stem cells in defined conditions. *Stem Cells* **31**, 941–952. (doi:10.1002/stem.1334)
54. Jones VC, Atkinson-Dell R, Verkhratsky A, Mohamet L. 2017 Aberrant iPSC-derived human astrocytes in Alzheimer's disease. *Cell Death Dis.* **8**, e2696. (doi:10.1038/cddis.2017.89)
55. Serio A *et al.* 2013 Astrocyte pathology and the absence of non-cell autonomy in an induced pluripotent stem cell model of TDP-43 proteinopathy. *Proc. Natl Acad. Sci. USA* **110**, 4697–4702. (doi:10.1073/pnas.1300398110)
56. Emdad L, D'Souza SL, Kothari HP, Qadeer ZA, Germano IM. 2012 Efficient differentiation of human embryonic and induced pluripotent stem cells into functional astrocytes. *Stem Cells Dev.* **21**, 404–410. (doi:10.1089/scd.2010.0560)
57. Gupta K *et al.* 2012 Human embryonic stem cell derived astrocytes mediate non-cell-autonomous neuroprotection through endogenous and drug-induced mechanisms. *Cell Death Differ.* **19**, 779–787. (doi:10.1038/cdd.2011.154)
58. Krencik R, Weick JP, Liu Y, Zhang ZJ, Zhang SC. 2011 Specification of transplantable astroglial subtypes from human pluripotent stem cells. *Nat. Biotechnol.* **29**, 528–534. (doi:10.1038/nbt.1877)
59. Livesey MR *et al.* 2016 Maturation and electrophysiological properties of human pluripotent stem cell-derived oligodendrocytes. *Stem Cells* **34**, 1040–1053. (doi:10.1002/stem.2273)
60. Mertens J, Marchetto MC, Bardy C, Gage FH. 2016 Evaluating cell reprogramming, differentiation and conversion technologies in neuroscience. *Nat. Rev. Neurosci.* **17**, 424–437. (doi:10.1038/nrn.2016.46)
61. Ehrlich M *et al.* 2017 Rapid and efficient generation of oligodendrocytes from human induced pluripotent stem cells using transcription factors. *Proc. Natl Acad. Sci. USA* **114**, E2243–E2252. (doi:10.1073/pnas.1614412114)
62. All AH *et al.* 2015 Early intervention for spinal cord injury with human induced pluripotent stem cells oligodendrocyte progenitors. *PLoS ONE* **10**, e0116933. (doi:10.1371/journal.pone.0116933)
63. Douvaras P, Wang J, Zimmer M, Hanchuk S, O'Bara MA, Sadiq S, Sim FJ, Goldman J, Fossati V. 2014 Efficient generation of myelinating oligodendrocytes from primary progressive multiple sclerosis patients by induced pluripotent stem cells. *Stem Cell Rep.* **3**, 250–259. (doi:10.1016/j.stemcr.2014.06.012)
64. Wang S *et al.* 2013 Human iPSC-derived oligodendrocyte progenitor cells can myelinate and rescue a mouse model of congenital hypomyelination. *Cell Stem Cell* **12**, 252–264. (doi:10.1016/j.stem.2012.12.002)

65. Hu BY, Du ZW, Zhang SC. 2009 Differentiation of human oligodendrocytes from pluripotent stem cells. *Nat. Protoc.* **4**, 1614–1622. (doi:10.1038/nprot.2009.186)
66. Tavian M, Péault B. 2005 Embryonic development of the human hematopoietic system. *Int. J. Dev. Biol.* **49**, 243–250. (doi:10.1387/ijdb.041957mt)
67. Monier A, Adle-Biassette H, Delezoide AL, Evrard P, Gressens P, Verney C. 2007 Entry and distribution of microglial cells in human embryonic and fetal cerebral cortex. *J. Neuropathol. Exp. Neurol.* **66**, 372–382. (doi:10.1097/nen.0b013e3180517b46)
68. Etemad S, Zamin RM, Ruitenberg MJ, Filgueira L. 2012 A novel *in vitro* human *microglia* model: characterization of human monocyte-derived microglia. *J. Neurosci. Methods* **209**, 79–89. (doi:10.1016/j.jneumeth.2012.05.025)
69. Abud EM *et al.* 2017 iPSC-derived human microglia-like cells to study neurological diseases. *Neuron* **94**, 278–293.e9. (doi:10.1016/j.neuron.2017.03.042)
70. Douvaras P *et al.* 2017 Directed differentiation of human pluripotent stem cells to microglia. *Stem Cell Rep.* **8**, 1516–1524. (doi:10.1016/j.stemcr.2017.04.023)
71. Pandya H *et al.* 2017 Differentiation of human and murine induced pluripotent stem cells to microglia-like cells. *Nat. Neurosci.* **20**, 753–759. (doi:10.1038/nn.4534)
72. Muffat J *et al.* 2016 Efficient derivation of microglia-like cells from human pluripotent stem cells. *Nat. Med.* **22**, 1358–1367. (doi:10.1038/nm.4189)
73. Muotri AR, Nakashima K, Toni N, Sandler VM, Gage FH. 2005 Development of functional human embryonic stem cell-derived neurons in mouse brain. *Proc. Natl Acad. Sci. USA* **102**, 18 644–18 648. (doi:10.1073/pnas.0509315102)
74. Patani R *et al.* 2012 Investigating the utility of human embryonic stem cell-derived neurons to model ageing and neurodegenerative disease using whole-genome gene expression and splicing analysis. *J. Neurochem.* **122**, 738–751. (doi:10.1111/j.1471-4159.2012.07825.x)
75. Cahan P, Daley GQ. 2013 Origins and implications of pluripotent stem cell variability and heterogeneity. *Nat. Rev. Mol. Cell Biol.* **14**, 357–368. (doi:10.1038/nrm3584)
76. Wiley LA, Anfinson KR, Cranston CM, Kaalberg EE, Collins MM, Mullins RF, Stone EM, Tucker BA. 2017 Generation of xeno-free, cGMP-compliant patient-specific iPSCs from skin biopsy. *Curr. Protoc. Stem Cell Biol.* **42**, 4a.12.1–4a.12.14. (doi:10.1002/cpsc.30)
77. Natalwala A, Kunath T. 2017 Preparation, characterization, and banking of clinical-grade cells for neural transplantation: scale up, fingerprinting, and genomic stability of stem cell lines. *Prog. Brain Res.* **230**, 133–150. (doi:10.1016/bs.pbr.2017.02.007)
78. Reidling JC *et al.* 2018 Human neural stem cell transplantation rescues functional deficits in R6/2 and Q140 Huntington's disease mice. *Stem Cell Rep.* **10**, 58–72. (doi:10.1016/j.stemcr.2017.11.005)