Back to the origins: Human brain organoids to investigate neurodegeneration

I. Faravelli[1], G. Costamagna[1], S. Tamanini, S. Corti[*]

Dino Ferrari Centre, Neuroscience Section, Department of Pathophysiology and Transplantation (DEPT), University of Milan, Neurology Unit, IRCCS Foundation Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy

HIGHLIGHTS

- Human organoids allow researchers to investigate brain development and pathology.
- Late disease onset could be linked to alterations during brain development.
- Brain organoids can be used to model neurodegenerative disorders.
- Organoid maturation can be enhanced by *in vivo* transplantation or *in vitro* patterning.

ARTICLE INFO

Keywords:
Brain organoids
Neurological disorders
Neurodegeneration
Disease modelling

ABSTRACT

Neurodegenerative disorders represent a high burden in terms of individual, social and economical resources. No ultimate therapy has been established so far; human brain morphology and development can not be entirely reproduced by animal models, and genomic, metabolic and biochemical differences might contribute to a limited predictive power for human translation. Thus, the development of human brain organoid models holds a wide potential to investigate the range of physiological and pathological features that characterise the early onset of the degeneration. Moreover, central nervous system development has gained a crucial role in the study of the pathogenesis of neurodegenerative disorders. Premature alterations during brain maturation have been related to late disease manifestations; genetic mutations responsible for neurodegeneration have been found in genes highly expressed during neural development. Elucidating the mechanisms triggering neuronal susceptibility to degeneration is crucial for pathogenetic studies and therapeutic discoveries. In the present work, we provide an overview on the current applications of human brain organoids towards studies of neurodegenerative diseases, with a survey on the recent discoveries and a closing discussion on the present challenges and future perspectives.

1. Introduction

Neurodegenerative diseases are a clinically and pathologically heterogeneous group of disorders that affects a specific subset of neurons and whose progression is nowadays inevitable (Przedborski et al., 2003). They can be classified according to the clinical phenotype or to the area most predominantly affected. Alzheimer's disease (AD), frontotemporal dementia (FTD), Parkinson's disease (PD) and Huntington's disease (HD) have a devastating impact on patients and families, representing a high burden in terms of individual, social and economical resources. No ultimate therapy has been established so far, although some of these conditions benefit the availability of drugs slightly able to modify the natural history. These disorders share some common features and pathogenetic mechanisms, which have been identified in protein misfolding and aggregation, altered RNA homeostasis, inflammation and involvement of non-neuronal cells and hindered lysosomal functioning (Katsnelson et al., 2016). No unique mechanism seems to be primarily causative of neurodegeneration, suggesting that the complex synergy of different pathways could play a role. Reliable human *in vitro* models are precious tools for the discovery of specific pathogenic mechanisms and potential therapeutic approaches.

Brain organoid cultures were implemented in 2013 by Lancaster

[*] Corresponding author at: Neuroscience Section, Department of Pathophysiology and Transplantation (DEPT), University of Milan, Neurology Unit, IRCCS Foundation Ca' Granda Ospedale Maggiore Policlinico, Via Francesco Sforza 35, 20122 Milan, Italy.
E-mail address: stefania.corti@unimi.it (S. Corti).
[1] These authors contributed equally to this work.

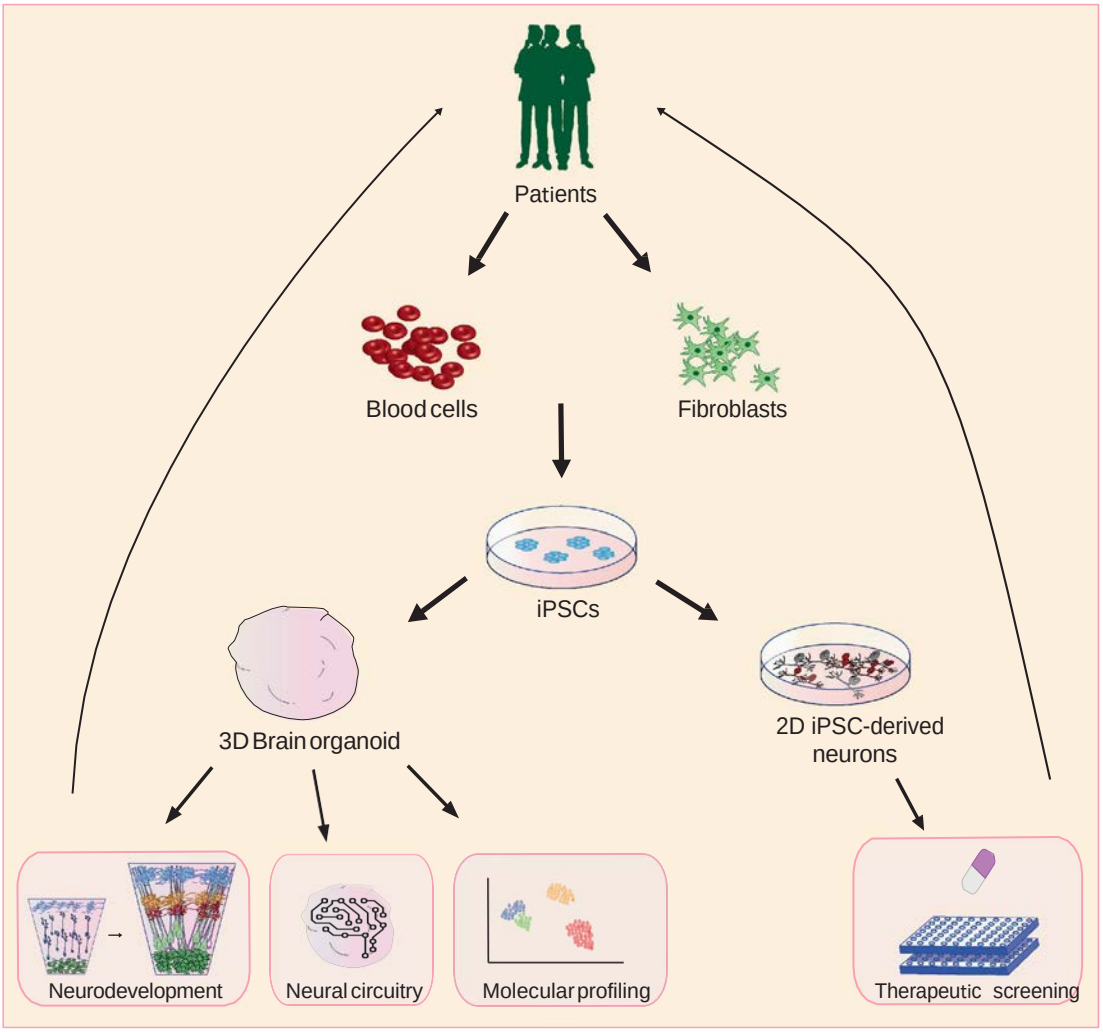

Fig. 1. Human induced stem cell-based models: 3D brain organoids vs 2D cultures. Human induced pluripotent stem cells (hIPS cells) can be reprogrammed from skin fibroblasts and blood cells. HIPS cells can then be differentiated into various cellular subtypes, including 2d neuronal monolayers, and into 3D brain organoids. While 2D based models can be used as high-throughput platforms for the screening of novel therapeutics, human brain organoids are versatile tools to investigate neurodevelopment, brain circuitry and conduct high-throughput molecular profiling.

and colleagues (Lancaster et al., 2013) offering a novel strategy to study brain development and disease. Lancaster's protocol generates neuroectoderm and subsequently neuroepithelium from embryoid bodies included in Matrigel droplets to provide an efficient scaffold for growth. These organoids contain cerebral cortex, plexi, meninges and retina, including the presence of discrete cellular populations in different and specific regions. They also display a rough organization in superficial ($Satb2^+$) and deep ($Ctip2^+$) layers, although not any stereotypical II-VI layers organization. Cell populations are interconnected in various circuitries and hold electric potential. Mature cortical neurons self-differentiate within the organoid and can be isolated and further cultured (Fig. 1). Many groups exploited different protocols to derive brain 3D cultures generating mature cortical neurons with postsynaptic receptors and spontaneously active neuronal networks that exhibit response to stimulation (Paşca et al., 2015; Quadrato et al., 2017; Rigamonti et al., 2016; Velasco et al., 2019).

Differentiation of patient derived induced pluripotent stem cells (iPSCs) lines allows researchers to produce patients' specific disease relevant cell types. Thus, iPSC derived organoids, which display a 3D spatial organization and complex cellular compartmentalization in addition to the possibility of surviving *in vitro* for many months, may be useful to investigate mechanisms involved in cell-to-cell spreading of misfolded protein aggregates and their dynamic evolution over time in neurodegenerative patients.

Moreover, the differentiation of human iPSCs into specific cell types, such as dopaminergic, glutamatergic, and GABAergic neuron, particularly if they could coexist in more complex 3D structures, would give the possibility to explore subtypes of neurons that are more vulnerable to the disease process. These studies can also be combined with single-cell RNA-seq analysis, which improve the ability to discriminate between neuronal subtypes according to their genetic signature. Elucidating the mechanisms triggering neuronal susceptibility to degeneration is crucial for pathogenetic studies and therapy discoveries. The possibility of generating many organoids in a relatively short time could enable the testing of compounds targeting specific pathogenic mechanisms.

Thus, the development of 3D brain organoid models holds a wide potential to investigate the range of physiological and pathological features that characterise the early onset of degeneration. Finally, it is worth considering that central nervous system development has gained a crucial role in the study of the pathogenesis of neurodegenerative disorders. Premature alterations during brain development have been related to late disease manifestations; genetic mutations responsible for neurodegeneration have been found in genes highly expressed during neural development (Marder and Mehler, 2012; Mehler, 2017).

In the present work, we provide an overview on the current

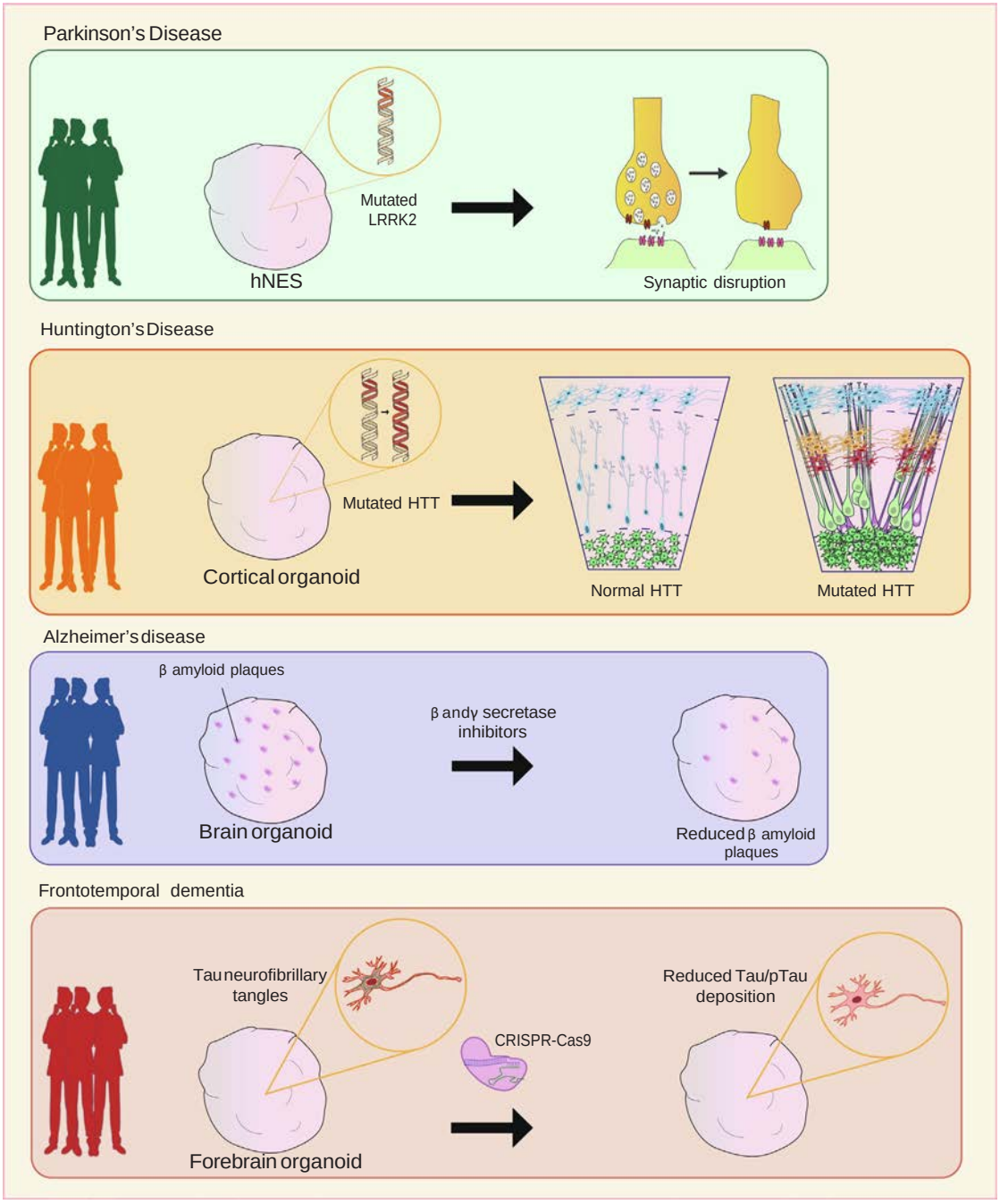

Fig. 2. Cellular and molecular phenotypes of common neurodegenerative disorders characterized using 3D human brain organoids. Advances in 3D stem cell research in the last decade have provided many new ways of studying neurodegeneration. The most investigated diseases are movement disorders such as Parkinson's and Huntington's diseases as well as different forms of dementia, including Alzheimer's disease and frontotemporal dementia. The possibility of integrating 3D brain organoids from human-derived iPSCs recapitulating the human fetal brain neurodevelopment with molecular biology techniques, e.g. CRISPR-cas, has opened new horizons to *in vitro* disease modeling resembling more closely their *in vivo* counterpart. At the same time, the protocol-based differentiation of neuroectodermal spheres into specific three-dimensional brain regions in the form of cortical, midbrain-like or forebrain organoids gives the opportunity to recapitulate more physiologically relevant cellular interactions and the cytoarchitectural dynamics of neurodegeneration.

applications of human brain organoids towards studies of neurodegenerative diseases (Fig. 2), with a survey on the recent discoveries and a closing discussion on the present challenges and future perspectives.

2. Movement disorders

Movement disorders are characterised by dysfunction of the movement programming resulting in alterations of voluntary movements and appearance of involuntary movements. They can be subdivided in hyperkinetic (mainly HD) or hypokinetic (mainly PD and Parkinson-plus syndromes). These disorders affect a group of subcortical neurons situated at the base of the forebrain, called basal ganglia, and functionally include striatum, globus pallidus, substantia nigra, subthalamic nucleus, as well as the red nucleus.

2.1. *Huntington's disease*

HD is a dominantly inherited neurodegenerative disorder caused by

Table 1
Experimental studies based on brain organoids for neurodegenerative disease modelling.

Cell line	Organoid type	Protocol	Days of differentiation	Disease	Phenotype	References
Human induced pluripotent stem cells	Neuroectodermal spheres	Modified from Oh et al., 2016	10	Lrrk2-related genetic Parkinson Disease	Altered expression of genes involved in synaptic transmission and vesicle trafficking	Son et al. (2017)
Human Induced pluripotent stem cells	Midbrain organoids	Modified from Jo et al., 2016	60	Lrrk2-related genetic Parkinson Disease	Decrease in dopaminergic neuronal markers, neurite length, increased susceptibility to MPTP-induced neurotoxicity, abnormal localization of phosphorylated α-synuclein in endosomes	Kim et al. (2019)
Human Induced pluripotent stem cells	Midbrain organoids	SB-431542, LDN-193189, CHIR99021, SAG, ROCK inhibitor, Ascorbic acid N2B27, SB	70	Lrrk2-related genetic Parkinson Disease	Reduction in the number and morphological complexity of dopaminergic neurons, neuromelanin inclusions	Smits et al. (2019)
Human Induced pluripotent stem cells	Cortical	Adapted Lancaster	105	Huntington's Disease	Immature ventricular/subventricular zone differentiation, altered cytoarchitecture and cortical layer organization, impaired genetic pathways related to neuronal migration and differentiation	Conforti et al. (2018)
Human Induced pluripotent stem cells	Brain	Modified Kadoshima Protocol, 2013	90–100	Familiar Alzheimer's Disease	Aβ and hyperphosphorylated tau protein aggregation, endosome abnormalities	Raja et al. (2016)
Human Induced pluripotent stem cells	Cortical	Modified Lancaster	110	Familiar Alzheimer's Disease	Aβ and tau protein aggregations, increased neuronal cell death	Gonzalez et al. (2018)
Human Induced pluripotent stem cells	Forebrain co-cultured with microglia cells	Raja Protocol, 2016	180	APOE4-related Alzheimer's Disease	Aβ and tau protein aggregation, altered microglia morphology, increased number of early endosomes	Lin et al. (2018)
Human Induced pluripotent stem cells	Forebrain	Raja Protocol, 2016	60	Frontotemporal dementia	Increased p25 and tau accumulation	Seo et al. (2017)

Overview of recent studies on the use of varying differentiation protocols to obtain brain region organoids. Starting from iPSCs derived from neurodegenerative patients, neural organoids have been derived and displayed specific pathological features. Aβ: amyloid beta, lrrk2: leucine-rich repeat kinase 2, MPTP: 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine.

Aβ: amyloid beta, lrrk2: leucine-rich repeat kinase 2, MPTP: 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine.

a CAG expansion mutation in the huntingtin gene on chromosome 4, resulting in a defective protein containing an expanded polyglutamine amino acid sequence. HD presentation is characterized by debilitating chorea, cognitive and psychiatric symptoms with no current available curative treatment. These symptoms are mainly related to neuronal loss in striatum and cortical areas. Whereas the adult form of HD shows a late fully symptomatic onset with a preclinical phase of heterogeneous duration, the juvenile form (also known as "Westphal variant"), due to a higher CAG repeats expansion, resembles a neurodevelopmental disorder. Research findings suggest that Huntingtin plays crucial role during early developmental phases in the organization of embryonic tissues, neurogenesis and cell cycle (Barnat et al., 2017; Conforti et al., 2018).

Both hiPSCs and 3D brain telencephalic organoids have been used as *in vitro* tools to evaluate neurodevelopmental defects linked to mHTT (Table 1). Compelling evidence suggests that mHTT leads to an abnormal acquisition of mature neuronal markers, with an increased abundance of immature cells if compared with control organoids (Conforti et al., 2018). Moreover, large CAG expansions correlate with complete disruption of the neuro-ectodermal formation process in mHTT hiPSCs (Conforti et al., 2018).

This feature is in line with previous publications, which showed that HTT is responsible for cortical neuronal polarity in HD mice models. HTT silencing during early neurodevelopment determines longer and more complicated dendritic outgrowth of cortical neurons from layer V and a reduction of dendritic length in neurons from layers II and III (Barnat et al., 2017; McKinstry et al., 2014). Moreover, HD hiPSCs-derived cortical neurons present shorter neurites compared to control cells (Mehta et al., 2018). Altogether, these cell morphological properties and tissue organizational features in both HD rodent models and 2D hiPSC-based cultures are in accordance with the disrupted cytoarchitecture and deficient cellular compartmentalization observed in 3D cultures. While gene-expression analyses showed that organoids from healthy individuals overlap with human fetal cortical areas, HD organoids displayed significant similarities with the immature ventricular zone/subventricular zone, suggesting a key role of the mHTT in slowing down fetal neurodevelopment. Supporting results were obtained in another 2D culture-based study focused on gene expression changes of HD hiPSC-derived striatal neurons, which were compared with those in rodent striatal tissue. Particularly, differentially expressed genes in the first group clustered with samples from the proliferative germinal zone, whereas non-disease samples clustered with those from the post-mitotic mantle zone (HD iPSC Consortium, 2017). Both 2D and 3D lines harboring mHTT may mature slower than non-disease lines or presenting an altered gene expression in earlier stages of development both in striatal and in cortical regions. These data are also in accordance with transcriptome analysis on HD hiPSCs-derived cortical neurons carried out by Metha et al., which showed a marked downregulation of genes associated with voltage-gated sodium currents (SCN1A, SCN2A, SCN4B, and SCN9A) if compared with control cells (Mehta et al., 2018). Considering the pivotal role played by these genes in generating a functional action potential, their downregulation could suggest a delayed maturation phenotype in cortical cultures, as previously described in HD iPSC-derived models of the developing striatum (HD iPSC Consortium, 2017; Mattis et al., 2015).

Furthermore, HD organoids showed a low expression of genes related to neuronal migration and differentiation such as SOX11, GAP 43 and CELSR3 (Conforti et al., 2018), similarly to the results obtained in another research on HD hiPSCs (The HD iPSC Consortium, 2012). It is worth noticing that some of the abovementioned data on tissue 3D spatial cytoarchitecture and cell compartmentalization can only be collected with the use of organoid-based protocols, a feature that could not be fully evaluated with 2D *in vitro* cultures. Nonetheless, it is still debatable whether these neurodevelopmental defects are relevant in the *in vivo* disease pathology and further investigations will be necessary to properly assess this correlation.

An interesting approach in HD research offered by 3D cultures and their long life-span potential *in vitro* is the possibility of studying later onset phenotypes with stressor or aging inducing agents, such as progerin (Guo et al., 2017). The possibility to recreate a more *in vivo* like microenvironment would have crucial implications for understanding the pathophysiology of neurodegenerative diseases such as HD.

2.2. Parkinson disease

PD is the second most frequent neurodegenerative disease following AD (Schwamborn, 2018). PD is caused by degeneration of dopaminergic neurons in the substantia nigra pars compacta (SNpc) (Dickson et al., 2009). Characteristic neuropathological findings in PD are Lewy's bodies, consisting of aggregates of misfolded proteins, such as α-synuclein, in neuronal bodies and processes.

Recent studies in PD stem cell research have described the generation and characterization of human midbrain-like organoids hMLO (Jo et al., 2016; Monzel et al., 2017; Smits et al., 2019; Tieng et al., 2014) (Table 1). HMLOs presented both glial cells (astrocytes and oligodendrocytes) and spatially patterned midbrain dopaminergic neurons (mDNs). MDNs were asymmetrically distributed in clusters, a critical feature mirroring the typical characteristic of the human brain, in which the soma of mDNs resides in the substantia nigra. In addition, hMLOs exhibited spontaneous electrophysiological activity of different subpopulations of neurons, including dopaminergic, GABAergic, and glutamatergic neurons (Smits et al., 2019). Neurons in hMLOs showed proper synapse formation and axonal myelination with the presence of nodes of Ranvier. Moreover, dopaminergic neurons expressed neuromelanin (NM), which is generally considered a neuroprotective factor deriving from metabolically highly active areas in midbrain substantia nigra and in noradrenergic neurons in locus ceruleus, the two brainstem areas most affected in PD (Fedorow et al., 2005; Jo et al., 2016). In parallel, NM released from damaged dopaminergic neurons could act as an endogenous activator of local midbrain microglia, which in turn increases local inflammation and neuronal toxicity that ultimately leads to neurodegeneration (Zhang et al., 2013). These results show how 3D cultures are valuable tools for generating organ-like structures *in vitro* presenting cytoarchitectural and functional features of their *in vivo* counterpart.

5 to10 % of all PD cases have a genetic background; while a minor part of them are *de novo* mutations, most of them are of familiar nature (Klein and Westenberger, 2012). Since the first mutated PD linked gene was discovered in 1997, (SNCA, encoding the protein alpha-synuclein), over a dozen others have been identified as causative genes of autosomal dominant or recessive mendelian forms of PD (Schwamborn, 2018). At the same time, many other genetic variants at risk loci seem to contribute to the disease (Sweet et al., 2015). Many of these genes have been investigated both in animal models and in human pluripotent stem cell (hPSCs) cultures (Schwamborn, 2018). The leucine-rich repeat kinase 2 (LRRK2) G2019S mutation is the most common genetic cause of familial Parkinson's disease (PD). A $LRKK2^{G2019S}$ mutation has been linked to an increased neurodegeneration (Winner et al., 2011) and to a decrease in neurite outgrowth (Godena et al., 2014). One of the main difficulties in understanding the role of LRRK2 in PD pathogenesis has been the development of models correctly recapitulating the LRRK2 mutant-associated disease phenotype. These experiments have been performed mainly in mice harboring this genetic mutation, failing to provide definitive evidence of progressive midbrain dopaminergic neurodegeneration and Lewy body formation as prominently as in the human brains, two main neuropathological features of the disease, (Chesselet et al., 2008; Giasson et al., 2002) or in 2D cultures (Xu et al., 2012). In the latter case, dopaminergic neurons differentiated from PD patient-derived hiPSCs harboring α -synuclein mutations, although showing a certain degree of disease-related neuronal toxicity, were generally immature (Chung et al., 2013).

In a recent study by Kim et al., isogenic $LRRK2^{G2019S}$- expressing

hMLO were generated from hiPSCs and recapitulated abnormal PD phenotypes in comparison with control organoids, revealing a decrease in dopaminergic neuronal markers TH, AADC, VMAT2 and DAT, a decrease in neurite length, an increased susceptibility to 1-methyl-4-phenyl-1.2.3.6-tetrahydropyridine (MPTP)-induced neurotoxicity and abnormal localization of phosphorylated α-synuclein in vescicular endosomal compartment. In another study by Smits et *al* it was possible to generate LRRK2^{G2019S} midbrain organoids presenting a decrease in number and complexity of dopaminergic neurons, which also occurs in PD patients' brain, reproducing a PD-relevant phenotype in a 3D environment (Kordower et al., 2013; Smits et al., 2019). Some of these pathological features could be reversed with the use of LRRK2 kinase inhibitor, suggesting that these 3D models can also be useful in the development of new therapies for PD (Kim et al., 2019). Furthermore, gene expression analysis showed an upregulated gene in LRRK2^{G2019S} midbrain organoids, the thioredoxin-interacting-protein (TXNIP), known to be associated with α-synuclein induced PD (Kim et al., 2019; Su et al., 2017). Importantly, gene set enrichment analysis revealed that differentially expressed gene set in LRRK2^{G2019S} organoids in comparison with LRRK2^{G2019S} 2D cultures was enriched in genes isolated from sporadic PD brain tissue, particularly TXNIP, demonstrating the unique role of 3D cultures in elucidating the molecular mechanisms underlying LRRK2^{G2019S}-related sporadic PD. An inhibition of TXNIP in midbrain organoids could rescue the LRRK2^{G2019S} associated phenotype with a reduction of α-synuclein aggregates, suggesting a potential role of TXNIP in LRRK2-associated sporadic PD and providing a functional connection between LRRK2 and α-synuclein thorough TXNIP in a 3D microenvironment. These results represent a proof of principle of the utility of 3D models in investigating PD pathophysiology.

Some researches point out that PD is not only a brain disease but is also characterized by a disruption of the brain-gut axis affecting the central and peripheral CNS as well as the autonomous enteric system (Liddle, 2018). Intestinal symptoms can precede neurological ones by many years (Schneider and Obeso, 2013). LRKK2 mutation has been linked to a disruption of both neuronal functions such as neurogenesis, mitochondrial and synaptic vesicular movement (Wang et al., 2012) and the intestinal microenvironment. In fact, genome-wide association analyses have documented how this gene can also contribute to inflammatory intestinal disorder, particularly Chron disease and ulcerative colitis (Abbott et al., 2001). An interesting approach has been based on gene expression analysis and signaling network in 3D human neuroectodermal spheres (hNESs) derived from LRKK2 patient-derived hiPSCs and human intestinal organoids (hIOs) (Son et al., 2017).

The gene expression profiles in LRRK2^{G2019S}-mutated hNESs and hIOs presented abnormalities if compared with wild-type controls (Son et al., 2017). In this study, transcriptome and microarray analysis showed that genes involved in synaptic vesicle trafficking and synaptic transmission such as RAB3A, SYT1, SYP, STX1B, SYN2 and SYN3, known to be disrupted in animal and 2D models of PD, (Migheli et al., 2013; Piccoli et al., 2011), were up-regulated in hNESs and hIOs carrying the mutation (Son et al., 2017). This study shows that 3D *in vitro* models with the same genetic background of PD patients can be promising tools for understanding the pathogenic effect of the mutation on cells from various tissues, such as neural and gastrointestinal cells, offering new insights on the pathophysiology of non-neurological manifestations observed in PD.

Further interesting researches could focus on hMLO models expressing mutated PD-related genes other than LRRK2 such as SNCA, whose genetic variants and duplications may possibly be involved in sporadic forms of PD (Hoffman-Zacharska et al., 2013). In this sense, it would be possible to use 3D-based platforms not only to study the rare genetic forms of PD, but also the more common sporadic ones. At same time, since some LRRK2^{G2019S} variants are linked to a reduced risk of PD onset, it would be interesting to investigate this protective effect on a cytoarchitectural and functional level with hMLO, evaluating the use of new potential therapeutic molecules on a cellular scale. Overall, although the modelling of neurodegenerative disorders with brain organoids is still at early stages, these results support the view that 3D *in vitro* models could offer unique insights into some pathophysiological mechanisms of PD.

3. Cognitive disorders

3.1. Alzheimer's disease

AD typically presents in patients older than 65 years with progressive memory loss, behavioral dysfunctions, language impairment. The main neuropathological features of AD are the extracellular accumulation of amyloid-β peptides and neurofibrillary tangles (NFT) composed of hyperphosphorylated tau protein (Serrano-Pozo et al., 2011). Tau protein is a microtubule-associated protein localized in the axon, which plays a crucial role in axonal transport by a process of microtubule stabilization. In AD, Tau protein translocates into the somatodendritic compartment and undergoes phosphorylation, in addition to misfolding, accumulation and formation of neurofibrillary tangles. (Serrano-Pozo et al., 2011) NFT are defined as intracellular inclusions of phosphorylated Tau (pTau) presenting a stereotypical pattern of spatiotemporal progression directly correlating with the cognitive decline (Arriagada et al., 1992). Aβ peptide, a 40 or 42 amino acid peptide derived from amyloid precursor protein (APP) after its cleavage by β- and γ-secretases, does not directly correlates with the cognitive decline and has a controversial role in physiological conditions (Hyman et al., 1993; Serrano-Pozo et al., 2011). Whereas most of the patients suffer from the sporadic form of the disease, 5% of all cases are associated with genetic mutations, particularly the early-onset and most aggressive familiar variants. These genetic forms involve Aβ processing enzymes such as presenilin 1 and 2 (*PSEN1*, *PSEN2*), known to be part of the γ-secretase complex, or mutations within or duplications of the APP gene itself (Choi et al., 2014).

It is possible to recapitulate the disease pathology to a certain extent with transgenic rodent models, which have offered invaluable insights into AD pathophysiology by overexpressing human genes related to familial AD (fAD). Unfortunately, they suffer from several limitations, among which the most critical one is the difficulty to reproduce NFT with Tau deposition (Sasaguri et al., 2017). Unless a human Tau transgene is inserted into the murine model, human PSEN and APP transgenic mice are unable to express protein Tau (Sasaguri et al., 2017). Furthermore, although rodents present many susceptibility genes for AD in their genomes, they do not develop AD-related phenotypes spontaneously, suggesting that some pathogenic trigger in humans may lack in the murine model (Kitazawa et al., 2012). Thus, alternative systems would be useful to study the disease pathogenesis of AD.

The use of 2D hPSCs derived from sporadic (sAD) and familiar fAD patients has paved the way to new *in vitro* disease models helping both to undercover the pathological mechanisms and to develop potential therapies (Choi and Tanzi, 2012). Different groups have performed tudies based on hPSCs-derived neural cells, but 2D monolayers from AD patients, even if able to express low level of toxic proteins, lack both amyloid plaques and tau tangles, limiting their potential use (Drummond and Wisniewski, 2017). Amyloid plaques accumulate in the extracellular compartment, disrupting the synaptic connections between cells, a disease hallmark that is lost in a two-dimensional environment (Raja et al., 2016).

To overcome these limitations, an interesting approach is represented by 3D *in vitro* cultures. A research investigating fAD mutations of APP and presenilin 1 showed extracellular deposition of amyloid-β and Tau tangles in a human embryonic stem cell-based 3D culture system (Choi et al., 2014). (Table 1). Moreover, Choi et al found that β- and γ-secretases inhibition induced a decrease in Tau accumulation, supporting the so called "amyloid hypothesis", according to which β-amyloid aggregation drives tauopathy (Choi et al., 2014;

Selkoe and Hardy, 2016). In another study, brain organoids from fAD patients harboring an APP mutation presented progressive amyloid accumulation in a time-dependent manner earlier than tau hyperphosphorylation. (Raja et al., 2016). In addition, AD patient-derived brain organoids displayed alterations in endosome morphology and recycling, with a higher number of larger endosomes, which can increase Aβ production by active cleavage of APP. This phenotype confirmed data already observed in 2D cultures and human brains (Cataldo et al., 2001; Israel et al., 2012; Raja et al., 2016; Toh and Gleeson, 2016).

In a recent research, cortical organoids (COs) derived from patients with different neurodegenerative diseases showed phenotypes typical of late-onset AD. Particularly, patient-derived fAD and Down syndrome (DS) COs' presented progressive accumulation of Aβ peptide in plaque-like structures with amylodogenic properties, in addition to phosphorylated Tau and neurofibrillary tangles, similarly to the ones observed in pathological human brains. These abnormalities were absent in control COs from healthy individuals, mouse embryonic stem cells, mouse pluripotent stem cells (PSCs) and Creutzfeld-Jakob patients (Gonzalez et al., 2018). Thus, brain organoids represent a powerful tool in order to recreate some key neuropathological features of AD *in vitro* and may be useful to unravel the causal and temporal relationships between the two main neuropathological components of the disease and to potentially evaluate novel therapies. In a recent study, Jorfi et al produced and validated a microarray system with human neurospheroids from fAD patient-derived stem cells presenting tau e amyloid aggregations, potentially useful in rapid compound screening (Jorfi et al., 2018).

Many researches focused on the role of neural cells in the genesis of Aβ cascade and Tau pathology in fAD models. However, the pathological aggregation may be partially linked to an impaired clearance, involving glial cells such as microglia and astrocytes in sAD (Hickman et al., 2008). Moreover, neurons as well as astrocytes and microglial cells express the APOE gene, the most important single genetic risk factor for sAD (Keren-Shaul et al., 2017; Kim et al., 2009). APOE allelic variants are associated with a different risk of disease onset. Indeed, APOE4 greatly increases AD risk relative to the *APOE3* and *APOE2* variants (Corder et al., 1993; Lambert et al., 2013). In a recent research, Lin et *al* tried to investigate the effect of APOE4 variant on microglia- and astrocyte-mediated Aβ clearance. They co-cultured hiPSCs-derived microglia-like cells expressing APOE4 allele with APOE4 forebrain organoids, generating the so-called "assembloids" (Lin et al., 2018). APOE4 microglial cells exhibited longer processes and decreased Aβ clearance. Long term cultures showing a full neuronal and astrocyte differentiation documented a time-dependent increase of Aβ aggregation, Tau accumulation and increased number of early endosomes in these cells compared with control organoids, establishing a reference for APOE-associated neuropathology of AD *in vitro* (Lin et al., 2018). Thus, this study shows how it is possible to incorporate both neurons and glial cells in 3D systems generating assembloids and to evaluate the impact of *APOE4* in sAD models providing a characterization of cell-type-specific changes induced by the mutation.

These studies demonstrate how 3D models represent a valuable tool for exploring the interplay between simultaneously developing neurons, microglia and macroglia in neurodegenerative diseases such as AD, where the spatio-temporal interaction between these cells seems to play a critical role (Abbott, 2018; Wendeln et al., 2018).

3.2. *Frontotemporal dementia*

Frontotemporal dementia (FTD) refers to a group of disorders with a progressive degeneration of frontal and/or temporal brain lobes (Bang et al., 2015). FTD accounts for 10–15% of all forms of dementia and is the second most frequent in patients younger than 65 years old after AD (Karageorgiou & Miller, 2014). Affected patients present with behavioral and/or language deficits (Bang et al., 2015; Burrell et al., 2016).

So far, no known treatment has been effective. The precise etiology of the disease remains unclear, though 40–50% of all FTD cases share a family history and are linked to different genetic mutations, such as Microtubuli Associated Protein Tau (MAPT), progranulin (GRN) and C9ORF72 (Bang et al., 2015). Experimental results obtained from different studies on FTD mice models have been so far contradictory. In fact, an inhibition of C9ORF72 gene expression induced by antisense oligonucleotides in mice brains determined no pathological or behavioral abnormalities (Lagier-Tourenne et al., 2013). Differently, in other studies a nucleotide expansion of C9ORF72 in mice caused neuronal loss and behavioral abnormalities similar to the ones in ALS-FTD syndromes (Chew et al., 2015). Therefore, actual rodent-based models seem not to be the most suited ones to explore the complex cytoarchitecture and cognitive abilities that are peculiar to the human brain.

The investigation of tau regulating proteins such as CDK5, a molecule whose function is increased by an aberrant p25 activity, has unraveled some pathological mechanisms underpinning FTD. Previous works suggested that an increase in CDK5 in association with p25 induced tau aggregation, while a reduction of its expression rescued neuronal degeneration (Kimura et al., 2014; Piedrahita et al., 2010). Particularly, MAPT mutation, the gene encoding protein tau, is linked to increased tau aggregation and neuronal toxicity in FTD models. Forebrain organoids obtained from FTD hiPSCs patients showed increased p25 levels (Seo et al., 2017). On the contrary, a CRIPSR-cas induced mutation of p35 inhibiting p25 conversion from p35 in 2 month old organoids induced decreased levels of tau and phosphorylated tau (Seo et al., 2017). Thus, FTD brain organoids proved to be powerful *in vitro* tools to evaluate the interplay between tau pathology and FTD (Table 1).

3.3. *Current pitfalls and future perspectives*

Brain organoids represent an unprecedented tool, closely resembling the human neural system organization, however they remain an *in vitro* model deprived of the entire variety of cell populations and environmental stimuli normally present in a whole embryo (Table 2). Indeed, available differentiation protocols do not allow to generate organoids growing beyond the equivalent of an early prenatal stage, which could represent a limitation for modelling pathologies that arise postnatally or during adulthood. Generating more mature organoids is hindered by the lack of vascularization into the neural tissue that should be spread enough to reach every cell into the packed organoid. Recently, Tabeke et al showed that organ buds generated *in vitro* from diverse tissues by combining specific progenitors with mesenchymal stem cells could promote organoid vascularization (Takebe et al., 2015). Another promising approach is represented by *in vivo*

Table 2
Limitations and perspectives of brain organoids.

Limitation	Potential Strategy
Absence of vascular bed	- Combined progenitors (mesenchymal and neural stem cells) - In vivo transplantation into rodent models
Absence of microglia	- Specific differentiation protocol - In vivo transplantation
Batch syndrome	- Patterned organoids - Modification of differentiation condition
Long-term maturation	- In vivo transplantation
Orientation	- Engineered scaffolds
Absence of outputs	- Organoid assembloids - In vivo transplantation
Late-onset phenotypes	- Stressor agents - Progerin-induced aging

3D organoids represent a fascinating novel tool to model development and disease; however, they are affected by a series of limitations that need to be overcome including high variability, the lack of orientation and a proper vascular bed.

transplantation of human brain organoids into adult mouse brain. This procedure prompts a vascularization that closely recapitulates steps occurring during developmental angiogenesis; Mansur et al were able to transplant cerebral organoids into a vascular niche inside the cortex of a mouse. Grafted organoids displayed typical germinal area and mature neurons and were extensively nourished by the host vascular bed (Mansour et al., 2018). Moreover, the presence of host microglia could be demonstrated inside the transplant. Indeed, microglia represents a population normally lacking inside brain organoids due to its different embryonic origin, but it appears to be important in the regulation of synaptic pruning and circuit development. Different protocols have been established to derive microglia *in vitro* starting from hPSCs, hinting to the possibility of combining it with brain organoid cultures at the early stages. Schwartz et al assembled neural precursors, mesenchymal stem cells, endothelial cells and microglia progenitors on a polyethylene scaffold to promote the growth of a complex network (Schwartz et al., 2015).

Very recently, Ormel et al showed that microglia could innately develop within cerebral organoids differentiated using Lancasters' protocol, without the dual-SMAD inhibition that is usually employed to induce neuroectoderm (Ormel et al., 2018). Organoid microglia showed a characteristic phenotype, was stained for specific markers and was able to prompt an inflammatory response with phagocytic ability. These results open the path to pathogenetic studies investigating the role of neuroinflammation into neuronal degeneration, a focus increasingly recognized in ALS and Alzheimer's disease.

One of the major limitations of the organoid method is its wide variability; several studies have described the model as highly heterogeneous (also referred as "batch syndrome"), in which different batches can display different behaviors in terms of quality and development (Kelava and Lancaster, 2016). This feature represents an obstacle to studies of toxicity or therapeutic screening and could be overcome by exploiting differentiation protocols towards more patterned organoids (i.e. cortical, ventral, dorsal or midbrain organoids) or finely tuning the starting number of plated cells and growth factor exploited (Quadrato et al., 2017; Velasco et al., 2019).

Brain organoids are deprived of structures that can provide orientation when they start differentiating; the advancement of bioengineering techniques provide interesting perspectives for the development of specific scaffold able to support and guide the growing tissue and that can be eventually selectively permeated by gradients of small molecules.

Finally, brain organoids lack inputs from the sensory system and motor outputs, which are crucial for a further circuit maturation; the possibility of organoid transplantation could in part overcome this obstacle offering a surrounding environment able to provide stimuli. A complementary approach is represented by the development of assembloids: organoids can be differentiated towards different regions of the nervous system and after that combined to form a unique interconnected structure (Sloan et al., 2018).

4. Conclusions

In this review, we provided an overview on the present possibilities and future challenges of using brain organoids to study neurodegenerative disorders. Despite fascinating progresses, the reliable generation of discrete and mature central nervous system regions represents a hurdle. New technologies are based on the integration of endothelial and immune populations inside organoids either transplanting them in a host nervous system or combining them *in vitro*. Moreover, scaffold and microfluidic technologies could offer further support in providing growth factors and environmental stimuli to pattern the neural tissue.

Neurodegenerative disorders have been traditionally correlated to the mechanisms of aging, but the central nervous system development has been increasingly studied in the pathogenesis of these conditions. Gene mutations responsible for neurodegenerative disorders have been related to genes extensively expressed during neural patterning, such as presenilin 1 and huntingtin. Thus, genetic mutations responsible for human diseases could hinder the physiological maturation of specific neural progenitors making them vulnerable to later environmental triggers. In this context, human organoids represent an unprecedented precious tool to deeply investigate brain development and pathology. Exploiting transcriptome profiling, they can be used to investigate the functional outcomes of specific genetic variations in combination with environmental triggers in determining neurodegenerative disorders. Progresses in establishing and refining maturation protocols could lead to the creation of organoid-based platforms to identify novel disease biomarkers and tailor novel personalized therapeutic approaches.

Acknowledgment

We gratefully thank the Associazione Centro Dino Ferrari for its support.

References

Abbott, A., 2018. Is 'friendly fire' in the brain provoking Alzheimer's disease? Nature 556, 426–428. https://doi.org/10.1038/d41586-018-04930-7.

Abbott, R.D., Petrovitch, H., White, L.R., Masaki, K.H., Tanner, C.M., Curb, J.D., Grandinetti, A., Blanchette, P.L., Popper, J.S., Ross, G.W., 2001. Frequency of bowel movements and the future risk of Parkinson's disease. Neurology 57, 456–462.

Arriagada, P.V., Marzloff, K., Hyman, B.T., 1992. Distribution of Alzheimer-type pathologic changes in nondemented elderly individuals matches the pattern in Alzheimer's disease. Neurology 42, 1681–1688. https://doi.org/10.1212/wnl.42.9.1681.

Bang, J., Spina, S., Miller, B.L., 2015. Frontotemporal dementia. Lancet 386, 1672–1682. https://doi.org/10.1016/S0140-6736(15)00461-4.

Barnat, M., Le Friec, J., Benstaali, C., Humbert, S., 2017. Huntingtin-Mediated Multipolar-Bipolar Transition of Newborn Cortical Neurons Is Critical for Their Postnatal Neuronal Morphology. Neuron 93, 99–114. https://doi.org/10.1016/J.NEURON.2016.11.035.

Burrell, J.R., Halliday, G.M., Kril, J.J., Ittner, L.M., Götz, J., Kiernan, M.C., Hodges, J.R., 2016. The frontotemporal dementia-motor neuron disease continuum. Lancet 388, 919–931. https://doi.org/10.1016/S0140-6736(16)00737-6.

Cataldo, A., Rebeck, G.W., Ghetti, B., Hulette, C., Lippa, C., Van Broeckhoven, C., Van Duijn, C., Cras, P., Bogdanovic, N., Bird, T., Peterhoff, C., Nixon, R., 2001. Endocytic disturbances distinguish among subtypes of alzheimer's disease and related disorders. Ann. Neurol. 50, 661–665. https://doi.org/10.1002/ana.1254.

Chesselet, M.-F., Fleming, S., Mortazavi, F., Meurers, B., 2008. Strengths and limitations of genetic mouse models of Parkinson's disease. Parkinsonism Relat. Disord. 14, S84–S87. https://doi.org/10.1016/J.PARKRELDIS.2008.04.004.

Chew, J., Gendron, T.F., Prudencio, M., Sasaguri, H., Zhang, Y.-J., Castanedes-Casey, M., Lee, C.W., Jansen-West, K., Kurti, A., Murray, M.E., Bieniek, K.F., Bauer, P.O., Whitelaw, E.C., Rousseau, L., Stankowski, J.N., Stetler, C., Daughrity, L.M., Perkerson, E.A., Desaro, P., Johnston, A., Overstreet, K., Edbauer, D., Rademakers, R., Boylan, K.B., Dickson, D.W., Fryer, J.D., Petrucelli, L., 2015. Neurodegeneration. C9ORF72 repeat expansions in mice cause TDP-43 pathology, neuronal loss, and behavioral deficits. Science 348, 1151–1154. https://doi.org/10.1126/science.aaa9344.

Choi, S.H., Kim, Y.H., Hebisch, M., Sliwinski, C., Lee, S., D'Avanzo, C., Chen, H., Hooli, B., Asselin, C., Muffat, J., Klee, J.B., Zhang, C., Wainger, B.J., Peitz, M., Kovacs, D.M., Woolf, C.J., Wagner, S.L., Tanzi, R.E., Kim, D.Y., 2014. A three-dimensional human neural cell culture model of Alzheimer's disease. Nature 515, 274–278. https://doi.org/10.1038/nature13800.

Choi, S.H., Tanzi, R.E., 2012. iPSCs to the rescue in Alzheimer's research. Cell Stem Cell 10, 235–236. https://doi.org/10.1016/j.stem.2012.02.011.

Chung, C.Y., Khurana, V., Auluck, P.K., Tardiff, D.F., Mazzulli, J.R., Soldner, F., Baru, V., Lou, Y., Freyzon, Y., Cho, S., Mungenast, A.E., Muffat, J., Mitalipova, M., Pluth, M.D., Jui, N.T., Schule, B., Lippard, S.J., Tsai, L.-H., Krainc, D., Buchwald, S.L., Jaenisch, R., Lindquist, S., 2013. Identification and rescue of -synuclein toxicity in parkinson patient-derived neurons. Science 80 (342), 983–987. https://doi.org/10.1126/science.1245296.

Conforti, P., Besusso, D., Bocchi, V.D., Faedo, A., Cesana, E., Rossetti, G., Ranzani, V., Svendsen, C.N., Thompson, L.M., Toselli, M., Biella, G., Pagani, M., Cattaneo, E., 2018. Faulty neuronal determination and cell polarization are reverted by modulating HD early phenotypes. Proc. Natl. Acad. Sci. U.S.A 115, E762–E771. https://doi.org/10.1073/pnas.1715865115.

Corder, E.H., Saunders, A.M., Strittmatter, W.J., Schmechel, D.E., Gaskell, P.C., Small, G.W., Roses, A.D., Haines, J.L., Pericak-Vance, M.A., 1993. Gene dose of apolipoprotein E type 4 allele and the risk of Alzheimer's disease in late onset families. Science 261, 921–923.

Dickson, D.W., Braak, H., Duda, J.E., Duyckaerts, C., Gasser, T., Halliday, G.M., Hardy, J., Leverenz, J.B., Del Tredici, K., Wszolek, Z.K., Litvan, I., 2009. Neuropathological assessment of Parkinson's disease: refining the diagnostic criteria. Lancet Neurol. 8, 1150–1157. https://doi.org/10.1016/S1474-4422(09)70238-8.

Drummond, E., Wisniewski, T., 2017. Alzheimer's disease: experimental models and reality. Acta Neuropathol. 133, 155–175. https://doi.org/10.1007/s00401-016-

1662-x.
Fedorow, H., Tribl, F., Halliday, G., Gerlach, M., Riederer, P., Double, K., 2005. Neuromelanin in human dopamine neurons: comparison with peripheral melanins and relevance to Parkinson's disease. Prog. Neurobiol. 75, 109–124. https://doi.org/10.1016/j.pneurobio.2005.02.001.
Giasson, B.I., Duda, J.E., Quinn, S.M., Zhang, B., Trojanowski, J.Q., Lee, V.M.-Y., 2002. Neuronal α-synucleinopathy with severe movement disorder in mice expressing A53T human α-synuclein. Neuron 34, 521–533. https://doi.org/10.1016/S0896-6273(02)00682-7.
Godena, V.K., Brookes-Hocking, N., Moller, A., Shaw, G., Oswald, M., Sancho, R.M., Miller, C.C.J., Whitworth, A.J., De Vos, K.J., 2014. Increasing microtubule acetylation rescues axonal transport and locomotor deficits caused by LRRK2 Roc-COR domain mutations. Nat. Commun. 5, 5245. https://doi.org/10.1038/ncomms6245.
Gonzalez, C., Armijo, E., Bravo-Alegria, J., Becerra-Calixto, A., Mays, C.E., Soto, C., 2018. Modeling amyloid beta and tau pathology in human cerebral organoids. Mol. Psychiatry 23, 2363–2374. https://doi.org/10.1038/s41380-018-0229-8.
Guo, W., Fumagalli, L., Prior, R., Van Den Bosch, L., 2017. Current advances and limitations in modeling ALS/FTD in a dish using induced pluripotent stem cells. Front. Neurosci. 11, 671. https://doi.org/10.3389/fnins.2017.00671.
HD iPSC Consortium, T.H. iPSC, 2017. Developmental alterations in Huntington's disease neural cells and pharmacological rescue in cells and mice. Nat. Neurosci. 20, 648–660. https://doi.org/10.1038/nn.4532.
Hickman, S.E., Allison, E.K., El Khoury, J., 2008. Microglial dysfunction and defective beta-amyloid clearance pathways in aging Alzheimer's disease mice. J. Neurosci. 28, 8354–8360. https://doi.org/10.1523/JNEUROSCI.0616-08.2008.
Hoffman-Zacharska, D., Koziorowski, D., Ross, O.A., Milewski, M., Poznański, J., Jurek, M., Wszolek, Z.K., Soto-Ortolaza, A., Sławek, J., Janik, P., Jamrozik, Z., Potulska-Chromik, A., Jasińska-Myga, B., Opala, G., Krygowska-Wajs, A., Czyżewski, K., Dickson, D.W., Bal, J., Friedman, A., 2013. Novel A18T and pA29S substitutions in α-synuclein may be associated with sporadic Parkinson's disease. Parkinsonism Relat. Disord. 19, 1057–1060. https://doi.org/10.1016/j.parkreldis.2013.07.011.
Hyman, B.T., Marzloff, K., Arrigada, P.V., 1993. The lack of accumulation of senile plaques or amyloid burden in Alzheimer's Disease suggests a dynamic balance between amyloid deposition and resolution. J. Neuropathol. Exp. Neurol. 52, 594–600. https://doi.org/10.1097/00005072-199311000-00006.
Israel, M.A., Yuan, S.H., Bardy, C., Reyna, S.M., Mu, Y., Herrera, C., Hefferan, M.P., Van Gorp, S., Nazor, K.L., Boscolo, F.S., Carson, C.T., Laurent, L.C., Marsala, M., Gage, F.H., Remes, A.M., Koo, E.H., Goldstein, L.S.B., 2012. Probing sporadic and familial Alzheimer's disease using induced pluripotent stem cells. Nature 482, 216–220. https://doi.org/10.1038/nature10821.
Jo, J., Xiao, Y., Sun, A.X., Cukuroglu, E., Tran, H.-D., Göke, J., Tan, Z.Y., Saw, T.Y., Tan, C.-P., Lokman, H., Lee, Y., Kim, D., Ko, H.S., Kim, S.-O., Park, J.H., Cho, N.-J., Hyde, T.M., Kleinman, J.E., Shin, J.H., Weinberger, D.R., Tan, E.K., Je, H.S., Ng, H.-H., 2016. Midbrain-like Organoids from Human Pluripotent Stem Cells Contain Functional Dopaminergic and Neuromelanin-Producing Neurons. Cell Stem Cell 19, 248–257. https://doi.org/10.1016/j.stem.2016.07.005.
Jorfi, M., D'Avanzo, C., Tanzi, R.E., Kim, D.Y., Irimia, D., 2018. Human neurospheroid arrays for in vitro studies of Alzheimer's disease. Sci. Rep. 8, 2450. https://doi.org/10.1038/s41598-018-20436-8.
Karageorgiou, E., Miller, B., 2014. Frontotemporal lobar degeneration: a clinical approach. Semin. Neurol. 34, 189–201. https://doi.org/10.1055/s-0034-1381735.
Katsnelson, A., De Strooper, B., Zoghbi, H.Y., 2016. Neurodegeneration: from cellular concepts to clinical applications. Sci. Transl. Med. 8, 364ps18. https://doi.org/10.1126/scitranslmed.aal2074.
Kelava, I., Lancaster, M.A., 2016. Dishing out mini-brains: current progress and future prospects in brain organoid research. Dev. Biol. 420, 199–209. https://doi.org/10.1016/j.ydbio.2016.06.037.
Keren-Shaul, H., Spinrad, A., Weiner, A., Matcovitch-Natan, O., Dvir-Szternfeld, R., Ulland, T.K., David, E., Baruch, K., Lara-Astaiso, D., Toth, B., Itzkovitz, S., Colonna, M., Schwartz, M., Amit, I., 2017. A unique microglia type associated with restricting development of Alzheimer's disease. Cell 169, 1276–1290.e17. https://doi.org/10.1016/j.cell.2017.05.018.
Kim, H., Park, H.J., Choi, H., Chang, Y., Park, H., Shin, J., Kim, J., Lengner, C.J., Lee, Y.K., Kim, J., 2019. Modeling G2019S-LRRK2 sporadic Parkinson's disease in 3D midbrain organoids. Stem Cell Rep. 12, 518–531. https://doi.org/10.1016/j.stemcr.2019.01.020.
Kim, J., Basak, J.M., Holtzman, D.M., 2009. The role of apolipoprotein E in Alzheimer's disease. Neuron 63, 287–303. https://doi.org/10.1016/j.neuron.2009.06.026.
Kimura, T., Ishiguro, K., Hisanaga, S., 2014. Physiological and pathological phosphorylation of tau by Cdk5. Front. Mol. Neurosci. 7, 65. https://doi.org/10.3389/fnmol.2014.00065.
Kitazawa, M., Medeiros, R., Laferla, F.M., 2012. Transgenic mouse models of Alzheimer disease: developing a better model as a tool for therapeutic interventions. Curr. Pharm. Des. 18, 1131–1147.
Klein, C., Westenberger, A., 2012. Genetics of Parkinson's disease. a008888 a8888. Cold Spring Harb. Perspect. Med. 2. https://doi.org/10.1101/cshperspect.a008888.
Kordower, J.H., Olanow, C.W., Dodiya, H.B., Chu, Y., Beach, T.G., Adler, C.H., Halliday, G.M., Bartus, R.T., 2013. Disease duration and the integrity of the nigrostriatal system in Parkinson's disease. Brain 136, 2419–2431. https://doi.org/10.1093/brain/awt192.
Lagier-Tourenne, C., Baughn, M., Rigo, F., Sun, S., Liu, P., Li, H.-R., Jiang, J., Watt, A.T., Chun, S., Katz, M., Qiu, J., Sun, Y., Ling, S.-C., Zhu, Q., Polymenidou, M., Drenner, K., Artates, J.W., McAlonis-Downes, M., Markmiller, S., Hutt, K.R., Pizzo, D.P., Cady, J., Harms, M.B., Baloh, R.H., Vandenberg, S.R., Yeo, G.W., Fu, X.-D., Bennett, C.F., Cleveland, D.W., Ravits, J., 2013. Targeted degradation of sense and antisense C9orf72 RNA foci as therapy for ALS and frontotemporal degeneration. Proc. Natl. Acad. Sci. U.S.A 110, E4530–E4539. https://doi.org/10.1073/pnas.1318835110.
Lambert, J.-C., Ibrahim-Verbaas, C.A., Harold, D., Naj, A.C., Sims, R., Bellenguez, C., Jun, G., DeStefano, A.L., Bis, J.C., Beecham, G.W., Grenier-Boley, B., Russo, G., Thornton-Wells, T.A., Jones, N., Smith, A.V., Chouraki, V., Thomas, C., Ikram, M.A., Zelenika, D., Vardarajan, B.N., Kamatani, Y., Lin, C.-F., Gerrish, A., Schmidt, H., Kunkle, B., Dunstan, M.L., Ruiz, A., Bihoreau, M.-T., Choi, S.-H., Reitz, C., Pasquier, F., Hollingworth, P., Ramirez, A., Hanon, O., Fitzpatrick, A.L., Buxbaum, J.D., Campion, D., Crane, P.K., Baldwin, C., Becker, T., Gudnason, V., Cruchaga, C., Craig, D., Amin, N., Berr, C., Lopez, O.L., De Jager, P.L., Deramecourt, V., Johnston, J.A., Evans, D., Lovestone, S., Letenneur, L., Morón, F.J., Rubinsztein, D.C., Eiriksdottir, G., Sleegers, K., Goate, A.M., Fiévet, N., Huentelman, M.J., Gill, M., Brown, K., Kamboh, M.I., Keller, L., Barberger-Gateau, P., McGuinness, B., Larson, E.B., Green, R., Myers, A.J., Dufouil, C., Todd, S., Wallon, D., Love, S., Rogaeva, E., Gallacher, J., St George-Hyslop, P., Clarimon, J., Lleo, A., Bayer, A., Tsuang, D.W., Yu, L., Tsolaki, M., Bossù, P., Spalletta, G., Proitsi, P., Collinge, J., Sorbi, S., Sanchez-Garcia, F., Fox, N.C., Hardy, J., Naranjo, M.C.D., Bosco, P., Clarke, R., Brayne, C., Galimberti, D., Mancuso, M., Matthews, F., Moebus, S., Mecocci, P., Del Zompo, M., Maier, W., Hampel, H., Pilotto, A., Bullido, M., Panza, F., Caffarra, P., Nacmias, B., Gilbert, J.R., Mayhaus, M., Lannfelt, L., Hakonarson, H., Pichler, S., Carrasquillo, M.M., Ingelsson, M., Beekly, D., Alvarez, V., Zou, F., Valladares, O., Younkin, S.G., Coto, E., Hamilton-Nelson, K.L., Gu, W., Razquin, C., Pastor, P., Mateo, I., Owen, M.J., Faber, K.M., Jonsson, P.V., Combarros, O., O'Donovan, M.C., Cantwell, L.B., Soininen, H., Blacker, D., Mead, S., Mosley, T.H., Bennett, D.A., Harris, T.B., Fratiglioni, L., Holmes, C., de Bruijn, R.F.A.G., Passmore, P., Montine, T.J., Bettens, K., Rotter, J.I., Brice, A., Morgan, K., Foroud, T.M., Kukull, W.A., Hannequin, D., Powell, J.F., Nalls, M.A., Ritchie, K., Lunetta, K.L., Kauwe, J.S.K., Boerwinkle, E., Riemenschneider, M., Boada, M., Hiltunen, M., Martin, E.R., Schmidt, R., Rujescu, D., Wang, L.-S., Dartigues, J.-F., Mayeux, R., Tzourio, C., Hofman, A., Nöthen, M.M., Graff, C., Psaty, B.M., Jones, L., Haines, J.L., Holmans, P.A., Lathrop, M., Pericak-Vance, M.A., Launer, L.J., Farrer, L.A., van Duijn, C.M., Van Broeckhoven, C., Moskvina, V., Seshadri, S., Williams, J., Schellenberg, G.D., Amouyel, P., 2013. Meta-analysis of 74,046 individuals identifies 11 new susceptibility loci for Alzheimer's disease. Nat. Genet. 45, 1452–1458. https://doi.org/10.1038/ng.2802.
Lancaster, M.A., Renner, M., Martin, C.-A., Wenzel, D., Bicknell, L.S., Hurles, M.E., Homfray, T., Penninger, J.M., Jackson, A.P., Knoblich, J.A., 2013. Cerebral organoids model human brain development and microcephaly. Nature 501, 373–379. https://doi.org/10.1038/nature12517.
Liddle, R.A., 2018. Parkinson's disease from the gut. Brain Res. 1693, 201–206. https://doi.org/10.1016/j.brainres.2018.01.010.
Lin, Y.-T., Seo, J., Gao, F., Feldman, H.M., Wen, H.-L., Penney, J., Cam, H.P., Gjoneska, E., Raja, W.K., Cheng, J., Rueda, R., Kritskiy, O., Abdurrob, F., Peng, Z., Milo, B., Yu, C.J., Elmsaouri, S., Dey, D., Ko, T., Yankner, B.A., Tsai, L.-H., 2018. APOE4 causes widespread molecular and cellular alterations associated with Alzheimer's disease phenotypes in human iPSC-derived brain cell types. Neuron 98, 1294. https://doi.org/10.1016/j.neuron.2018.06.011.
Mansour, A.A., Gonçalves, J.T., Bloyd, C.W., Li, H., Fernandes, S., Quang, D., Johnston, S., Parylak, S.L., Jin, X., Gage, F.H., 2018. An in vivo model of functional and vascularized human brain organoids. Nat. Biotechnol. 36, 432–441. https://doi.org/10.1038/nbt.4127.
Marder, K., Mehler, M.F., 2012. Development and neurodegeneration: turning HD pathogenesis on its head. Neurology 79, 621–622. https://doi.org/10.1212/WNL.0b013e3182648bfe.
Mattis, V.B., Tom, C., Akimov, S., Saeedian, J., Østergaard, M.E., Southwell, A.L., Doty, C.N., Ornelas, L., Sahabian, A., Lenaeus, L., Mandefro, B., Sareen, D., Arjomand, J., Hayden, M.R., Ross, C.A., Svendsen, C.N., 2015. HD iPSC-derived neural progenitors accumulate in culture and are susceptible to BDNF withdrawal due to glutamate toxicity. Hum. Mol. Genet. 24, 3257–3271. https://doi.org/10.1093/hmg/ddv080.
McKinstry, S.U., Karadeniz, Y.B., Worthington, A.K., Hayrapetyan, V.Y., Ozlu, M.I., Serafin-Molina, K., Risher, W.C., Ustunkaya, T., Dragatsis, I., Zeitlin, S., Yin, H.H., Eroglu, C., 2014. Huntingtin is required for normal excitatory synapse development in cortical and striatal circuits. J. Neurosci. 34, 9455–9472. https://doi.org/10.1523/JNEUROSCI.4699-13.2014.
Mehler, M.F., 2017. Shining a light on early stress responses and late-onset disease vulnerability. Proc. Natl. Acad. Sci. 114, 2109–2111. https://doi.org/10.1073/pnas.1700323114.
Mehta, S.R., Tom, C.M., Wang, Y., Bresee, C., Rushton, D., Mathkar, P.P., Tang, J., Mattis, V.B., 2018. Human Huntington's disease iPSC-derived cortical neurons display altered transcriptomics, morphology, and maturation. Cell Rep. 25, 1081–1096.e6. https://doi.org/10.1016/j.celrep.2018.09.076.
Migheli, R., Del Giudice, M.G., Spissu, Y., Sanna, G., Xiong, Y., Dawson, T.M., Dawson, V.L., Galioto, M., Rocchitta, G., Biosa, A., Serra, P.A., Carri, M.T., Crosio, C., Iaccarino, C., 2013. LRRK2 affects vesicle trafficking, neurotransmitter extracellular level and membrane receptor localization. PLoS One 8, e77198. https://doi.org/10.1371/journal.pone.0077198.
Monzel, A.S., Smits, L.M., Hemmer, K., Hachi, S., Moreno, E.L., van Wuellen, T., Jarazo, J., Walter, J., Brüggemann, I., Boussaad, I., Berger, E., Fleming, R.M.T., Bolognin, S., Schwamborn, J.C., 2017. Derivation of human midbrain-specific organoids from neuroepithelial stem cells. Stem Cell Rep. 8, 1144–1154. https://doi.org/10.1016/j.stemcr.2017.03.010.
Ormel, P.R., Vieira de Sá, R., van Bodegraven, E.J., Karst, H., Harschnitz, O., Sneeboer, M.A.M., Johansen, L.E., van Dijk, R.E., Scheefhals, N., Berdenis van Berlekom, A., Ribes Martínez, E., Kling, S., MacGillavry, H.D., van den Berg, L.H., Kahn, R.S., Hol, E.M., de Witte, L.D., Pasterkamp, R.J., 2018. Microglia innately develop within cerebral organoids. Nat. Commun. 9, 4167. https://doi.org/10.1038/s41467-018-06684-2.
Paşca, A.M., Sloan, S.A., Clarke, L.E., Tian, Y., Makinson, C.D., Huber, N., Kim, C.H., Park,

J.-Y., O'Rourke, N.A., Nguyen, K.D., Smith, S.J., Huguenard, J.R., Geschwind, D.H., Barres, B.A., Paşca, S.P., 2015. Functional cortical neurons and astrocytes from human pluripotent stem cells in 3D culture. Nat. Methods 12, 671–678. https://doi.org/10.1038/nmeth.3415.

Piccoli, G., Condliffe, S.B., Bauer, M., Giesert, F., Boldt, K., De Astis, S., Meixner, A., Sarioglu, H., Vogt-Weisenhorn, D.M., Wurst, W., Gloeckner, C.J., Matteoli, M., Sala, C., Ueffing, M., 2011. LRRK2 controls synaptic vesicle storage and mobilization within the recycling pool. J. Neurosci. 31, 2225–2237. https://doi.org/10.1523/JNEUROSCI.3730-10.2011.

Piedrahita, D., Hernández, I., López-Tobón, A., Fedorov, D., Obara, B., Manjunath, B.S., Boudreau, R.L., Davidson, B., Laferla, F., Gallego-Gómez, J.C., Kosik, K.S., Cardona-Gómez, G.P., 2010. Silencing of CDK5 reduces neurofibrillary tangles in transgenic alzheimer's mice. J. Neurosci. 30, 13966–13976. https://doi.org/10.1523/JNEUROSCI.3637-10.2010.

Przedborski, S., Vila, M., Jackson-Lewis, V., 2003. Neurodegeneration: what is it and where are we? J. Clin. Invest. 111, 3–10. https://doi.org/10.1172/JCI17522.

Quadrato, G., Nguyen, T., Macosko, E.Z., Sherwood, J.L., Min Yang, S., Berger, D.R., Maria, N., Scholvin, J., Goldman, M., Kinney, J.P., Boyden, E.S., Lichtman, J.W., Williams, Z.M., McCarroll, S.A., Arlotta, P., 2017. Cell diversity and network dynamics in photosensitive human brain organoids. Nature 545, 48–53. https://doi.org/10.1038/nature22047.

Raja, W.K., Mungenast, A.E., Lin, Y.-T., Ko, T., Abdurrob, F., Seo, J., Tsai, L.-H., 2016. Self-organizing 3D human neural tissue derived from induced pluripotent stem cells recapitulate Alzheimer's disease phenotypes. PLoS One 11, e0161969. https://doi.org/10.1371/journal.pone.0161969.

Rigamonti, A., Repetti, G.G., Sun, C., Price, F.D., Reny, D.C., Rapino, F., Weisinger, K., Benkler, C., Peterson, Q.P., Davidow, L.S., Hansson, E.M., Rubin, L.L., 2016. Large-scale production of mature neurons from human pluripotent stem cells in a three-dimensional suspension culture system. Stem Cell Rep. 6, 993–1008. https://doi.org/10.1016/j.stemcr.2016.05.010.

Sasaguri, H., Nilsson, P., Hashimoto, S., Nagata, K., Saito, T., De Strooper, B., Hardy, J., Vassar, R., Winblad, B., Saido, T.C., 2017. APP mouse models for Alzheimer's disease preclinical studies. EMBO J. 36, 2473–2487. https://doi.org/10.15252/embj.201797397.

Schneider, S.A., Obeso, J.A., 2013. Movement disorders: improved understanding of early disease. Lancet Neurol. 12, 10–12. https://doi.org/10.1016/S1474-4422(12)70302-2.

Schwamborn, J.C., 2018. Is Parkinson's disease a neurodevelopmental disorder and will brain organoids help us to understand It? Stem Cells Dev. 27, 968–975. https://doi.org/10.1089/scd.2017.0289.

Schwartz, M.P., Hou, Z., Propson, N.E., Zhang, J., Engstrom, C.J., Costa, V.S., Jiang, P., Nguyen, B.K., Bolin, J.M., Daly, W., Wang, Y., Stewart, R., Page, C.D., Murphy, W.L., Thomson, J.A., 2015. Human pluripotent stem cell-derived neural constructs for predicting neural toxicity. Proc. Natl. Acad. Sci. 112, 12516–12521. https://doi.org/10.1073/pnas.1516645112.

Selkoe, D.J., Hardy, J., 2016. The amyloid hypothesis of Alzheimer's disease at 25 years. EMBO Mol. Med. 8, 595–608. https://doi.org/10.15252/emmm.201606210.

Seo, J., Kritskiy, O., Watson, L.A., Barker, S.J., Dey, D., Raja, W.K., Lin, Y.-T., Ko, T., Cho, S., Penney, J., Silva, M.C., Sheridan, S.D., Lucente, D., Gusella, J.F., Dickerson, B.C., Haggarty, S.J., Tsai, L.-H., 2017. Inhibition of p25/Cdk5 Attenuates Tauopathy in Mouse and iPSC Models of Frontotemporal Dementia. J. Neurosci. 37, 9917–9924. https://doi.org/10.1523/JNEUROSCI.0621-17.2017.

Serrano-Pozo, A., Frosch, M.P., Masliah, E., Hyman, B.T., 2011. Neuropathological alterations in Alzheimer disease. Cold Spring Harb. Perspect. Med. 1, a006189. https://doi.org/10.1101/cshperspect.a006189.

Sloan, S.A., Andersen, J., Paşca, A.M., Birey, F., Paşca, S.P., 2018. Generation and assembly of human brain region–specific three-dimensional cultures. Nat. Protoc. 13, 2062–2085. https://doi.org/10.1038/s41596-018-0032-7.

Smits, L., Magni, S., Grzyb, K., MA Antony, P., Krueger, R., Skupin, A., Bolognin, S., Schwamborn, J., 2019. Single-cell transcriptomics reveals multiple neuronal cell types in human midbrain-specific organoids. https://doi.org/10.1101/589598.

Smits, L.M., Reinhardt, L., Reinhardt, P., Glatza, M., Monzel, A.S., Stanslowsky, N., Rosato-Siri, M.D., Zanon, A., Antony, P.M., Bellmann, J., Nicklas, S.M., Hemmer, K., Qing, X., Berger, E., Kalmbach, N., Ehrlich, M., Bolognin, S., Hicks, A.A., Wegner, F., Sterneckert, J.L., Schwamborn, J.C., 2019b. Modeling Parkinson's disease in midbrain-like organoids. NPJ Park. Dis. 5, 5. https://doi.org/10.1038/s41531-019-0078-4.

Son, M.-Y., Sim, H., Son, Y.S., Jung, K.B., Lee, M.-O., Oh, J.-H., Chung, S.-K., Jung, C.-R., Kim, J., 2017. Distinctive genomic signature of neural and intestinal organoids from familial Parkinson's disease patient-derived induced pluripotent stem cells. Neuropathol. Appl. Neurobiol. 43, 584–603. https://doi.org/10.1111/nan.12396.

Su, C.-J., Feng, Y., Liu, T.-T., Liu, X., Bao, J.-J., Shi, A.-M., Hu, D.-M., Liu, T., Yu, Y.-L., 2017. Thioredoxin-interacting protein induced α-synuclein accumulation via inhibition of autophagic flux: implications for Parkinson's disease. CNS Neurosci. Ther. 23, 717–723. https://doi.org/10.1111/cns.12721.

Sweet, E.S., Saunier-Rebori, B., Yue, Z., Blitzer, R.D., 2015. The Parkinson's Disease-Associated Mutation LRRK2-G2019S Impairs Synaptic Plasticity in Mouse Hippocampus. J. Neurosci. 35, 11190–11195. https://doi.org/10.1523/JNEUROSCI.0040-15.2015.

Takebe, T., Enomura, M., Yoshizawa, E., Kimura, M., Koike, H., Ueno, Y., Matsuzaki, T., Yamazaki, T., Toyohara, T., Osafune, K., Nakauchi, H., Yoshikawa, H.Y., Taniguchi, H., 2015. Vascularized and Complex Organ Buds from Diverse Tissues via Mesenchymal Cell-Driven Condensation. Cell Stem Cell 16, 556–565. https://doi.org/10.1016/j.stem.2015.03.004.

The HD iPSC Consortium, 2012. Induced pluripotent stem cells from patients with Huntington's disease show CAG-repeat-expansion-associated phenotypes. Cell Stem Cell 11, 264–278. https://doi.org/10.1016/J.STEM.2012.04.027.

Tieng, V., Stoppini, L., Villy, S., Fathi, M., Dubois-Dauphin, M., Krause, K.-H., 2014. Engineering of midbrain organoids containing long-lived dopaminergic neurons. Stem Cells Dev. 23, 1535–1547. https://doi.org/10.1089/scd.2013.0442.

Toh, W.H., Gleeson, P.A., 2016. Dysregulation of intracellular trafficking and endosomal sorting in Alzheimer's disease: controversies and unanswered questions. Biochem. J. 473, 1977–1993. https://doi.org/10.1042/BCJ20160147.

Velasco, S., Kedaigle, A.J., Simmons, S.K., Nash, A., Rocha, M., Quadrato, G., Paulsen, B., Nguyen, L., Adiconis, X., Regev, A., Levin, J.Z., Arlotta, P., 2019. Individual brain organoids reproducibly form cell diversity of the human cerebral cortex. Nature 570, 523–527. https://doi.org/10.1038/s41586-019-1289-x.

Wang, X., Yan, M.H., Fujioka, H., Liu, J., Wilson-Delfosse, A., Chen, S.G., Perry, G., Casadesus, G., Zhu, X., 2012. LRRK2 regulates mitochondrial dynamics and function through direct interaction with DLP1. Hum. Mol. Genet. 21, 1931–1944. https://doi.org/10.1093/hmg/dds003.

Wendeln, A.-C., Degenhardt, K., Kaurani, L., Gertig, M., Ulas, T., Jain, G., Wagner, J., Häsler, L.M., Wild, K., Skodras, A., Blank, T., Staszewski, O., Datta, M., Centeno, T.P., Capece, V., Islam, M.R., Kerimoglu, C., Staufenbiel, M., Schultze, J.L., Beyer, M., Prinz, M., Jucker, M., Fischer, A., Neher, J.J., 2018. Innate immune memory in the brain shapes neurological disease hallmarks. Nature 556, 332–338. https://doi.org/10.1038/s41586-018-0023-4.

Winner, B., Melrose, H.L., Zhao, C., Hinkle, K.M., Yue, M., Kent, C., Braithwaite, A.T., Ogholikhan, S., Aigner, R., Winkler, J., Farrer, M.J., Gage, F.H., 2011. Adult neurogenesis and neurite outgrowth are impaired in LRRK2 G2019S mice. Neurobiol. Dis. 41, 706–716. https://doi.org/10.1016/j.nbd.2010.12.008.

Xu, Q., Shenoy, S., Li, C., 2012. Mouse models for LRRK2 Parkinson's disease. Parkinsonism Relat. Disord. 18, S186–S189. https://doi.org/10.1016/S1353-8020(11)70058-X.

Zhang, W., Zecca, L., Wilson, B., Ren, H.-W., Wang, Y.-J., Wang, X.-M., Hong, J.-S., 2013. Human neuromelanin: an endogenous microglial activator for dopaminergic neuron death. Front. Biosci. (Elite Ed) 5, 1–11.