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In and out: Benchmarking *in vitro*, *in vivo*, *ex vivo*, and xenografting approaches for an integrative brain disease modeling pipeline

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SUMMARY

Human cellular models and their neuronal derivatives have afforded unprecedented advances in elucidating pathogenic mechanisms of neuropsychiatric diseases. Notwithstanding their indispensable contribution, animal models remain the benchmark in neurobiological research. In an attempt to harness the best of both worlds, researchers have increasingly relied on human/animal chimeras by xenografting human cells into the animal brain. Despite the unparalleled potential of xenografting approaches in the study of the human brain, literature resources that systematically examine their significance and advantages are surprisingly lacking. We fill this gap by providing a comprehensive account of brain diseases that were thus far subjected to all three modeling approaches (transgenic rodents, *in vitro* human lineages, human-animal xenografting) and provide a critical appraisal of the impact of xenografting approaches for advancing our understanding of those diseases and brain development. Next, we give our perspective on integrating xenografting modeling pipeline with recent cutting-edge technological advancements.

INTRODUCTION

Brain development is a spatially and temporally tightly regulated process of gene expression (Silbereis et al., 2016). The increasingly sophisticated brain organization and function makes it highly vulnerable, so that even subtle dysregulations can result in neurological impairments that can become evident early or later on in life (i.e., neurodevelopmental and neurodegenerative disorders).

The advent of somatic cell reprogramming, involving the reverting of a terminally differentiated cell to a pluripotent stage by viral expression of a handful of genes (Takahashi and Yamanaka, 2006), was a watershed moment for the disease modeling field, chiefly because it provided, for the first time, an unlimited source of previously inaccessible, relevant cell types. More recently, the emergence of increasingly sophisticated 3D cellular models (i.e., brain organoids) has promised to revolutionize disease modeling and make the study of the brain experimentally more tractable.

Despite the tremendous contribution that cellular *in vitro* models have made in elucidating disease mechanisms (Adamo et al., 2015; Brennand et al., 2011; Eiraku et al., 2008; Lancaster et al., 2013; López-Tobón et al., 2023; Paşca et al., 2011), the *in vivo* animal models remain the gold standard, especially when it comes to assessment of behavioral readouts and neurobiological disease mechanisms *in vivo*, and are widely used for preclinical validation of drug efficacy and safety, in spite of recent major, transformative changes that authorize the use of cell-based assays and computer-based models for assessing safety and effectiveness of novel drugs (Jucker, 2010; Nestler and Hyman, 2010; Vandamme, 2014; Wadman, 2023; Wax, 1995; FDA Modernization Act 2.0, 2022). In an attempt to combine *in vitro* with *in vivo* approaches and harness their edges, researchers have xenografted human cellular models into the rodent brain (Espuny-Camacho et al., 2013; Gaspard et al., 2008; Mansour et al., 2018; Neuhof, 1923; Revah et al., 2022). Notwithstanding the transformative potential of human-rodent chimeras for uncovering the underlying mechanisms of brain structure and function in health and disease, resources that systematically evaluate their significance and their potential integration with relevant cutting-edge technologies from neighboring fields are lacking, thus hindering the adoption of xenografting approaches by the wider scientific community.

In the first part of this review, we provide a systematic account of the brain diseases that were thus far subjected to all three kinds of modeling approaches (transgenic

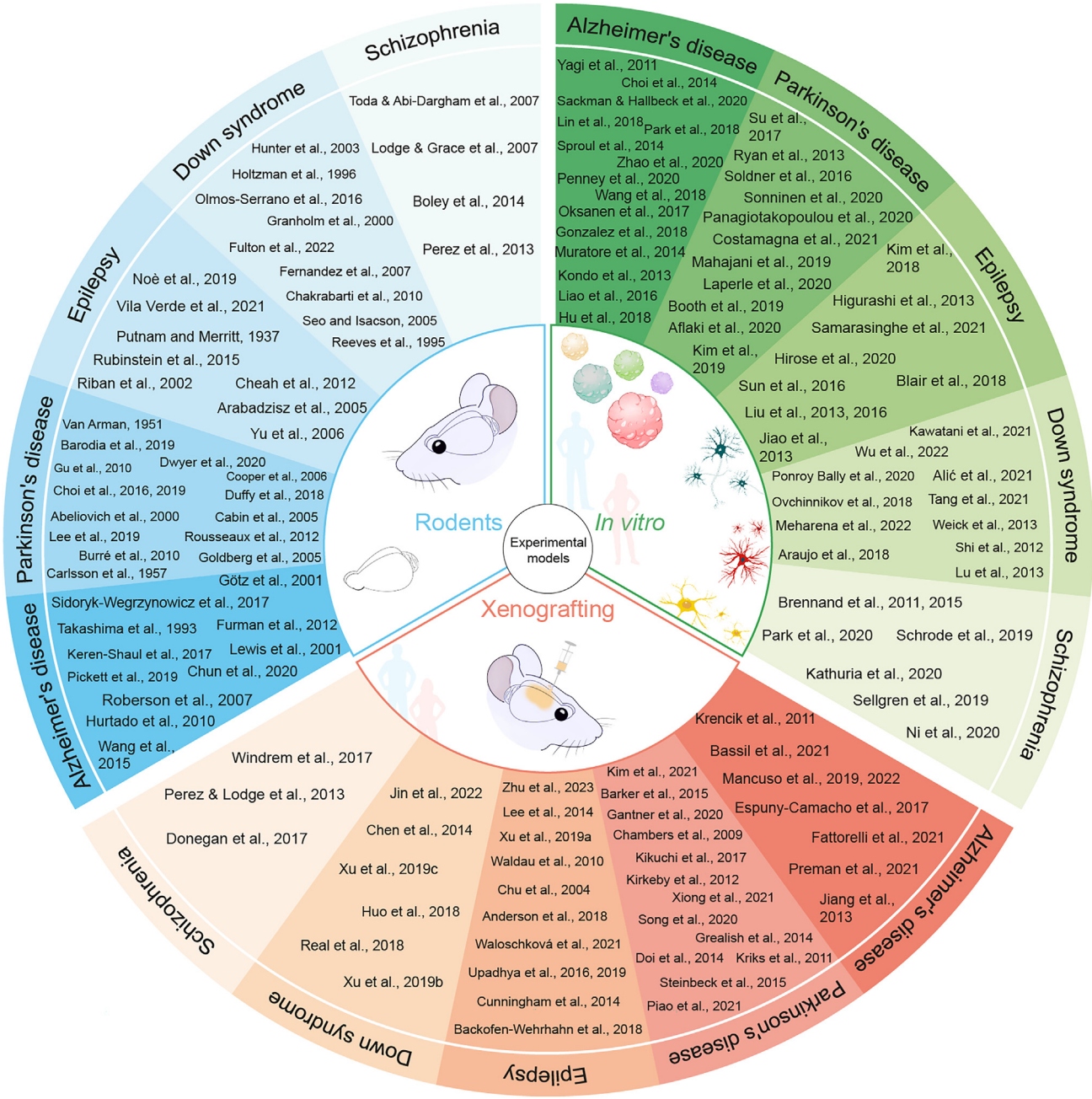

Figure 1. Brain disorders modeled using *in vitro*, *in vivo*, and xenografting approaches

List of brain disorders investigated using all three modeling approaches and representative references for each disorder. The list of brain disorders includes neurodegenerative (Alzheimer's and Parkinsons' disease), neurodevelopmental (Down syndrome and Schizophrenia), and common neurological disorders (Epilepsy).

rodents, *in vitro* human neural lineages, and human-rodent xenografting) and provide a critical appraisal of the impact of human-rodent xenografting approaches for advancing our knowledge of those diseases and brain development. Focusing on paradigmatic cases, the juxtaposition of findings from the three models cross-illuminates their specificities and defines the edge provided by a xenograft-centered roadmap for the systematic *in vivo* benchmarking of *in vitro* human neural models (Figures 1 and 2).

Building on the lessons learned from these initial paradigms, in the second part we put the xenografting

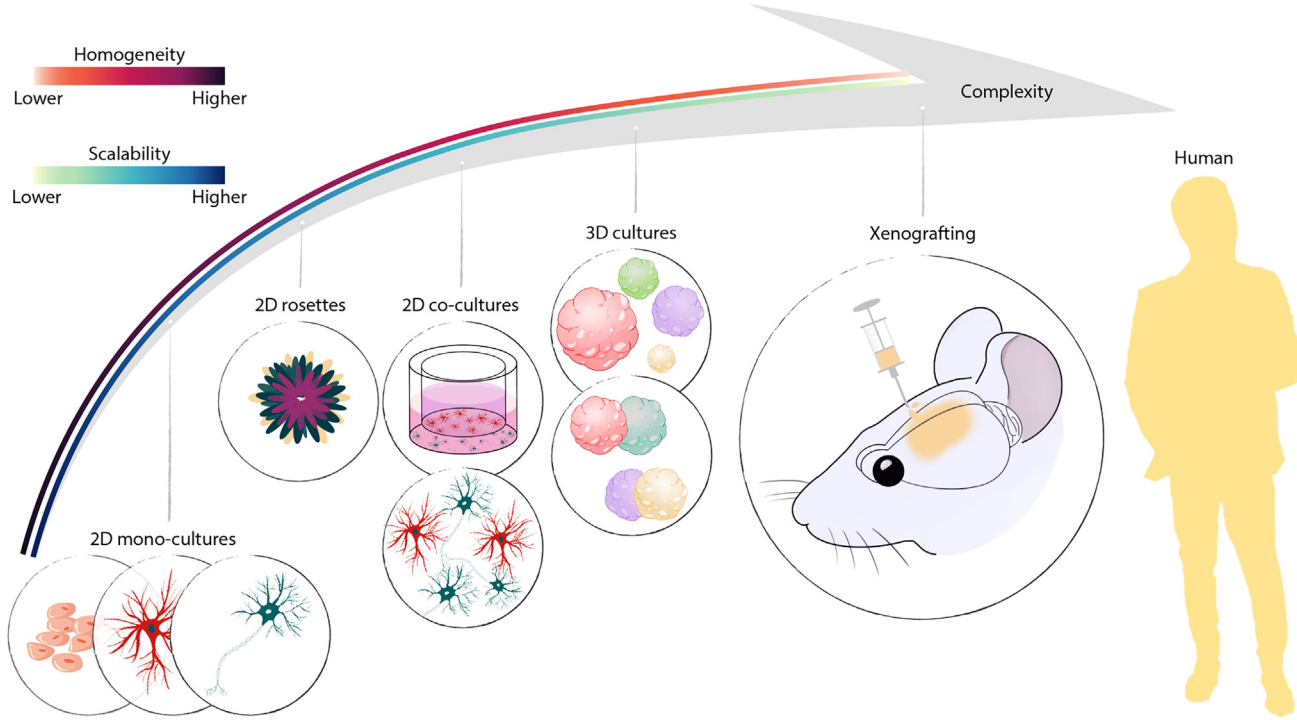

Figure 2. Modeling approaches for brain disorders

2D cellular culture systems due to their reductionistic nature permit an unparalleled degree of scalability that gradually diminishes with more sophisticated, multi-dimensional systems. In contrast, complexity is high in 3D and xenografting systems and low in simple cellular models.

modeling pipeline into the context of cutting-edge technological advancements that, together, are best poised to bring such models to full fruition since they afford unprecedented opportunity to interrogate human neural tissue within the organismal and behaving context of the most advanced rodent models.

XENOGRAFTING APPROACHES ELUCIDATING NEURODEVELOPMENT AND NEURONAL DIFFERENTIATION

Early studies with embryonic or induced pluripotent stem cells (iPSCs) utilized xenografting to probe the competence of stem cells to differentiate and recapitulate *in vivo* neurodevelopmental milestones. An early prominent example was the generation of glutamatergic induced neurons (iNs) from embryonic cells or iPSC by overexpression of the proneural transcription factor neurogenin-2; iN functionally integrated into the adult mouse striatum by forming extensive neuronal arborizations and synaptic connections (Zhang et al., 2013). Similarly, ectopic expression of a cocktail of 5 transcription factors efficiently converted mouse and human fibroblasts into GABAergic neurons. Transplantation into mouse hippocampus showed that gamma-aminobutyric acid (GABA) iN not only established robust synaptic contacts but also efficiently integrated into the host circuitry as shown by patch-clamp recordings and optogenetics approaches (Colasante et al., 2015). Work from Vanderhaeghen laboratory utilized extensively xenografting approaches to demonstrate that human and mouse embryonic stem cells (ESCs) and human iPSC (hiPSC) are inherently capable of recapitulating cardinal aspects of *in vivo* neuronal differentiation and corticogenesis. Remarkably, when mouse ESCs were grafted into the mouse neonatal brain, they developed extensive projections targeting specific cortical regions, revealing an intrinsically programmed cortical identity without dictation from the host brain (Gaspard et al., 2008). Similarly, grafted human ESC (hESC) and iPSC into the mouse cortex developed reciprocal synaptic connections with the host circuitries even receiving thalamic input, demonstrating an unprecedented functional integration. Importantly, human stem cells revealed a species-specific neuronal maturation potential as they reached full maturity at 9 months after transplantation, closely matching temporally the maturation of human neurons *in vivo*, without the influence of the mouse host (Espuny-Camacho et al., 2013).

Stem cells hold an enormous potential for brain repair. This was elegantly exemplified by xenografting mouse and hESCs into the mouse adult cortex (Espuny-Camacho et al., 2018; Falkner et al., 2016; Michelsen et al., 2015). Following chemical lesion of the adult visual cortex, ESCs were either grafted directly or specified first *in vitro* to differentiate into pyramidal neurons of occipital cortex identity and then grafted into the lesioned area. Over months, grafted neurons reestablished the lost connectivity of lesioned neurons by developing reciprocal long-range axonal projections, received area-specific afferent input, and efficiently integrated into the damaged circuits as shown by responses to light stimulation making them indistinguishable from host cortical neurons (Espuny-Camacho et al., 2018; Falkner et al., 2016; Michelsen et al., 2015). Worth noting that, in some adult models, the successful restoration of lost connectivity required lesioning, the *a priori* fate specification of ESCs *in vitro*, and a match between donor and host area identity. Notwithstanding, more recently it became apparent that when grafted into the neonatal mouse cortex, hESCs still retain the capacity for functional integration even in the absence of lesioning and cortical area match between donor and host. Specifically, hESCs grafted into the ventricles of neonatal mice matured over several months, exhibiting the donor species-specific protracted temporal maturation. Grafted neurons exhibited progressive morphological and electrophysiological maturation, gradually stabilizing their dendritic spines dynamics, indications of complete, functional, and reciprocal neuronal connections. Remarkably, the grafted mature neurons showcased an unprecedented functional integration propensity into the host visual cortex circuits by demonstrating robust and tuned visually driven responses to moving bars of different directions and frequencies, strikingly similar to endogenous mouse cortical neurons (Linaro et al., 2019). All the aforementioned studies used glutamatergic pyramidal neurons; nevertheless, complete, mature neuronal circuits contain also inhibitory interneurons, which are also critical for defining the critical period of visual acuity (Fagiolini and Hensch, 2000). Although not xenografting, embryonic mouse medial ganglionic eminence (MGE)-derived interneurons grafted into the visual cortex of visually deprived adult mice integrated into the appropriate visual cortex circuitries as shown by their participation to visually evoked responses. Strikingly, the grafting reinstituted the critical period in adulthood and improved visual acuity in previously visually deprived adult mice (Davis et al., 2015).

Xenografting approaches have proved extremely relevant even with the most recent 3D brain organoids models. Despite their transformative potential, brain organoids lack an efficient circulatory system for the even distribution of nutrients and oxygen, invariably leading to necrotic areas and ectopic activation of cellular stress responses, which in turn might prevent the appearance of diverse neuronal subtypes and brain fidelity (Bhaduri et al., 2020; Di Lullo and Kriegstein, 2017). All these aspects that have plagued the nascent field from the very beginning were resolved upon organoid grafting into the mouse brain. As a matter of fact, hESC-derived brain organoids were differentiated *in vitro* for 40–50 days and subsequently transplanted into the brain of immunocompromised adult mice. Similar to 2D neuronal cultures, grafted organoid-derived neurons showed progressive maturation, established reciprocal connections with the host neurons, and integrated into the host neuronal circuits as shown by calcium imaging and optogenetics experiments. Critically, the neuronal apoptosis in the organoid core was dramatically reduced, and functional blood vessels innervated the human organoids (Mansour et al., 2018). Likewise, upon transplantation in the mouse cortex, organoid-derived neurons stably downregulated expression of metabolic stress markers and increased progenitor and juvenile neuronal subtype specification (Bhaduri et al., 2020), dramatically enhancing their fidelity to the developing human brain. Very recent studies have started to redefine the horizon of xenografting approaches potential for disease modeling (Revah et al., 2022; Schafer et al., 2023). Grafting of intact organoids into newborn rats' somatosensory cortex ensured graft's integration with the host circuit without disrupting the endogenous developmental trajectories. Grafted organoids grew over months, and their neurons developed more mature morphological, synaptic, and electrophysiological features compared with their *in vitro* counterparts. Remarkably, maturation and functional integration of grafted organoids with the development of reciprocal connections modulated complex behaviors such as reward seeking and learning (Revah et al., 2022). The aforementioned studies highlight the tremendous advancements in our understanding of neuronal differentiation and neuronal circuitries formation that xenografting models have afforded us and which were not previously possible in neither purely *in vitro* nor animal *in vivo* models. This new knowledge grounds the hopes for the implementation of xenografting approaches in the modeling toolbox for the study of complex neurodevelopmental disorders that so far have been notoriously challenging to model.

BRAIN DISORDERS MODELED USING *IN VITRO*, *IN VIVO*, AND XENOGRAFTING APPROACHES

Alzheimer's disease

Already in the early 1900s, the use of postmortem tissue allowed neuropathologist Alois Alzheimer to observe degenerating neurons with bundles of fibrils and senile

plaques scattered over the cortex (Alzheimer et al., 1995; Yamaguchi et al., 1988). Alzheimer's disease (AD), the most common cause of dementia worldwide, is a tau pathology characterized by extracellular deposits of amyloid beta (Aβ), the main component of amyloid plaques and tau-containing intracellular neurofibrillary tangles, which in turn are caused by the hyperphosphorylation of the microtubule protein tau and its dissociation from the microtubules (Alzheimer et al., 1995; Braak and Braak, 1991; Khachaturian, 1985). Despite their inherent limitation of only providing a retrospective inference prone to several confounders and incompatibility for establishing causation, studies from postmortem brains of AD patients have revealed valuable features. These include the potential to diagnose the cause of death, quantify cellular and molecular markers of several neuronal processes, and retrospectively analyze and assess the heterogeneity of AD patients (Braak and Braak, 1991; McCullumsmith et al., 2014; Stan et al., 2006). However, data derived from human disease modeling *in vitro* tools as well as from animal models provide specific mechanistic and behavioral insights that cannot be generated using postmortem tissue.

A number of transgenic animal models have been developed to recapitulate specific pathological features of AD and have provided important insights into the disease (LaFerla and Green, 2012). Cleavage of amyloid precursor protein (APP) by β and γ secretases produces Aβ aggregates and formation of soluble oligomers, which are toxic to the neurons because they adversely affect synaptic structure and function and cellular homeostasis (Lambert et al., 1998; Walsh et al., 2002). The fact that Aβ toxicity is affected by the levels of tau was discovered using transgenic mice models, including i) P301S mutant tau (PS19) Tg mouse model of the four-repeat microtubule-associated protein tau-generating animals with conditional (inducible/reversible) tau and ii) bigenic models by crossing PS19 Tg mouse model with the well-known PDAPP model that overexpresses mutant V717F APP, among other relevant hybrid transgenic rodent models (Götz et al., 2001; Hurtado et al., 2010; Lewis et al., 2001; Pickett et al., 2019; Roberson et al., 2007; Takashima et al., 1993). The relationship between Aβ toxicity and tau levels has been confirmed more recently *in vitro* using iPSC-derived neurons (Hu et al., 2018; Penney et al., 2020; Sackmann and Hallbeck, 2020) and cerebral organoids (Gonzalez et al., 2018). All these highlight a rare instance of convergence between postmortem studies, transgenic animals, and human cellular models in revealing the complex interplay between Aβ aggregates and tau and suggest that integrative experimental systems are better suited for delineating human pathophysiology.

The involvement of non-neuronal cell types of the CNS in the worsening of AD pathology, namely astrocytes, was another important contribution of animal models, although their critical role was already recognized by Alzheimer (1910). It has been established that astrocytes are not merely supportive cells in the CNS (see review Araque et al., 1999) and that astrocytic pathology at earlier stages of AD disrupts synaptic connectivity, affects neurotransmitter homeostasis, and induces neuronal death in mouse models, contributing to the earliest neuronal deficits observed in AD (Furman et al., 2012; Sidoryk-Wegrzynowicz et al., 2017). Additionally, studies using human and mice models have shown that, at later stages, astrocytes become reactive, leading to other downstream consequences associated with disease progression, and contributing to the inflammatory component of neurodegeneration (Chun et al., 2020; Liao et al., 2016; Oksanen et al., 2017). The critical role of astrocytes in AD neuronal pathology is another point of convergence between human and mouse model studies and underscores the value of combining experimental approaches. Yet, recent findings call for caution as major differences between human and mouse astrocytes are emerging. A systematic comparison between human and mouse astrocytes, and particularly their responses to hypoxia, oxidative stress, and inflammation, revealed major species-specific differences in transcriptional regulation, mitochondrial physiology, and ability to detoxify. Grafting of human astrocytes into the mouse brain enabled a more in-depth comparison and showed that human-specific transcriptional regulation is intrinsically programmed and minimally affected by neurons and other brain cell types (Li et al., 2021). Besides astrocytes, microglia, a type of neuroglia that act as resident macrophages in the brain, have also been linked to AD neurodegeneration. Reactive microgliosis, a term encompassing the changes in morphology and function of microglia following CNS insult, and increased apoptotic rates are features commonly observed around amyloid plaques in both Aβ-transgenic and tauopathy mice models (Wang et al., 2015; Yoshiyama et al., 2007). It is still not fully understood whether microglial functions in AD are beneficial but not sufficient or whether are effective at early stages but lose their efficiency or even become obsolete in the irreversibly damaged brain. Intense efforts to characterize microglia cell types and functional states using omics at single-cell resolution enabled the recognition of disease-associated microglia (DAMs). DAMs subtypes, distinct from homeostatic microglia, were identified by single-cell RNA sequencing in 5xfFAD mouse model of Aβ accumulation and exhibit transcriptional signatures of genes expressed by classical macrophages, as well as modules for the interferon response, stress response, lysosomal function, and lipid metabolism (Keren-Shaul et al., 2017). Although the relationship between glial cells and AD was observed in several postmortem specimens (Itagaki et al., 1989),

additional recent studies motivated the community to challenge the neuron-centric view, even more so as glia (astrocytes and microglia) are the first line of defense in the CNS against any acute or chronic non-physiological perturbation, especially in neurodegenerative disorders (Dräger et al., 2022; Habib et al., 2020; Liddelow et al., 2017; Soreq et al., 2017; Xu et al., 2019b).

These distinctive properties of glia were recently exemplified in a landmark study by Schafer and colleagues. They colonized brain organoids with human PSC (hPSC)-derived microglia and upon transplantation into immunocompromised mice observed the gradual molecular and functional maturation of human microglia and their direct involvement in brain's immune defense and reaction to environmental insults. To highlight the potential of this xenografting *in vivo* platform for disease modeling, the authors revealed brain-microglia crosstalk in autism and an autism-specific microglia phenotype (Schafer et al., 2023). Although applied in an autism model, these reports clearly illustrate the edges of xenografting approaches *vis-à-vis* animal models or human *in vitro* models, in particular, for revealing human-specific microglia properties in health and disease *in vivo*.

A series of studies in the last decade, highlighted that iPSCs, despite their age and neuronal maturation-related limitations, can recapitulate hallmarks of AD neuropathology. Initial studies aimed at uncovering the impact of specific AD mutations on hiPSC-derived neurons and evaluated the efficacy of drug compounds. For instance, neurons from patients with familial AD (fAD) caused by duplication of *APP* gene, *E693Δ*, or *APPV717I* mutations contained more pathological markers, including higher accumulation of amyloid-β, phosphotau, glycogen synthase kinase-3β (GSK-3β), and endosomes, as well as oxidative stress when compared with sporadic AD patients, suggesting a convergence on molecular pathways of different AD-relevant mutations. Interestingly, treatment with β- but not γ-secretase inhibitor has been shown to be effective in reducing phosphotau and aGSK-3β levels, whereas docosahexaenoic acid was efficacious in reducing oxidative stress (Kondo et al., 2013; Muratore et al., 2014). Furthermore, neural progenitors (Sproul et al., 2014) and neurons from patients with mutations in presenilins 1 and 2 (PSEN) that cause fAD secrete more amyloid β42 (Aβ42), and that could be remedied by γ-secretase inhibitors (Yagi et al., 2011). Mutations in *PSEN1* affect also astrocytes derived from patients' iPSC by increasing levels of β-amyloid accumulation, altered cytokine release, and disruption of calcium homeostasis (Oksanen et al., 2017), providing strong evidence for the contribution of non-neuronal cells in AD. The critical roles of astrocytes and microglia in AD were further corroborated by examining the impact of apolipoprotein E4 (*APOE4*), the strongest risk factor gene for AD, on different brain cell types (Lin et al., 2018). Transcriptional profiling of astrocytes revealed defects on β-amyloid uptake and lipid metabolism, whereas microglia, similarly to astrocytes, exhibited less efficient β-amyloid uptake and disruption of inflammatory response, while at the same time transcriptional profiling and biochemical analysis of neurons exhibited accelerated neuronal differentiation, increased synaptic density and secretion of Aβ42, higher tau phosphorylation, and GABAergic neurons degeneration (Lin et al., 2018; Wang et al., 2018). *APOE4* effects were specific and dependent on its structural conformation, as the alleviation of the aforementioned defects was possible by either conversion of *APOE4* to *APOE3* or treatment with the *APOE4* structure corrector PH002 (Lin et al., 2018; Wang et al., 2018). Interestingly, most of the aforementioned AD neuropathological phenotypes were also recapitulated in 3D cellular models. As such, the detrimental effect of *APOE4* on synaptic density, apoptosis, and high levels of β-amyloid were replicated recently in cerebral organoids from AD patients (Zhao et al., 2020). Choi and colleagues (Choi et al., 2014) used a Matrigel-based 3D human model to show that fAD mutations, unlike in previous 2D models, could recapitulate, in addition to abnormal high levels of β-amyloid and phosphotau and the presence of β-amyloid plaques. The abnormally high levels of β-amyloid and phosphotau and the accumulation of neurofibrillary tangles-like aggregates were also recapitulated in cerebral organoids generated from patients with fAD and intriguingly from Down syndrome (DS) patients (Gonzalez et al., 2018). On the other hand, Park and colleagues found microglia recruitment, neurotoxic phenotypes, and oxidative stress that was detrimental to neurons and astrocytes (Park et al., 2018), using a microfluidic platform to examine the interaction of neurons, astrocytes, and microglia with AD-relevant mutations, in a 3D physiological environment.

It has become apparent that the advent of cell reprogramming and iPSCs advanced dramatically our understanding of AD pathophysiology and provided a high-throughput platform for the testing of promising therapeutic compounds in human cellular models. Nevertheless, it is still not known whether and to what degree the cellular phenotypes associated with AD have actually discernible impact on cognition and behavioral readouts.

To address how astrocytes influence AD-associated phenotypes, Preman and colleagues xenografted hiPSC-derived astrocyte progenitors into the brains of neonatal mice. The authors documented that the engrafted cells not only displayed human-specific morphology and functionally integrated into the rodent host but also under pathological conditions underwent morphological changes, showing hypertrophic and atrophic phenotypes

in response to amyloid plaques (Preman et al., 2021). Interestingly, other studies have focused on studying how human iPSC-derived neurons are affected upon exposure to murine AD models. For that purpose, after Espuny-Camacho et al. transplanted human neurons into the brains of newborn mice, they observed that cells matured and integrated into the brain and, following several structural and functional alterations, underwent neurodegeneration (Espuny-Camacho et al., 2017). On the other hand, additional efforts in optimizing the current protocols for engrafting hiPSC-derived microglial cells are still ongoing (Bassil et al., 2021; Fattorelli et al., 2021; Jiang et al., 2013; Krencik et al., 2011; Mancuso et al., 2019). For instance, microglial cells successfully engrafted and matured within the mouse brains and were able to respond to trauma revealing human-specific genes involved in the control of inflammation that had never been described before in rodent microglial cells (Hasselmann et al., 2019). More recently, preliminary data showed that grafted human microglia had a gradual response to amyloid-β pathology that differs from mouse microglia toward the same insult and proposed that microglia follow distinct activation routes that might directly affect the disease course in unpredicted ways (preprint, Mancuso et al., 2022). These studies highlighted species-specific features that can only be captured using human cellular models to reconstruct human-specific responsive genes/networks and distinct cell states of microglia in response to amyloid-β plaques.

For AD in particular, the integration of *in vitro* human cellular models with animal models enabled i) functional validation of cellular and molecular phenotypes uncovered previously in either purely *in vitro* human or AD animal models, ii) a unique opportunity to distinguish human-specific aspects of AD neuropathology, iii) better understanding of AD disease mechanisms by confirming the contribution of glial cells, and iv) a translational platform for predictive and personalized drug development.

Parkinson's disease

Parkinson's disease (PD) is characterized by progressive loss of dopaminergic (DA) neurons of the *substantia nigra pars compacta* (SNpc), depletion of dopamine levels, and accumulation of intracellular aggregates, mainly composed by α-synuclein (SNCA), named Lewy bodies (LBs) (Bernheimer et al., 1973; Ehringer and Hornykiewicz, 1960; Polymeropoulos et al., 1997; Spillantini et al., 1997). Although PD clinical features typically include resting tremor, muscle rigidity, bradykinesia, and impaired posture, several other non-motor manifestations arise while PD gradually worsens in severity, including depression, cognitive dysfunction, and insomnia (Gelb et al., 1999; Levin et al., 1989; Nausieda et al., 1982; Tandberg et al., 1996). While motor symptoms are clinically detectable, the brain pathology can only be confirmed by examining postmortem tissues (Raunio et al., 2019; Saito et al., 2003).

One of the key discoveries in the field was the use of L-dopa (L-3,4-dihydroxy phenylalanine), the most commonly prescribed medicine as well as the gold standard of drug treatment for PD. The discovery of the catecholamine metabolism in rat brains was a turning point in PD research and ushered in the era of L-dopa clinical trials in PD patients (Carlsson et al., 1957; Fahn et al., 2004; Van Arman, 1951; Yahr et al., 1969). A plethora of animal models have enabled both molecular- and cellular-level investigations into the mechanisms of action and development of L-dopa-induced motor complications to further explore PD variability and improve its treatment (see review Cenci and Crossman, 2018) and furthermore have been instrumental for investigating the role of specific PD genes. To date, approximately 20 genes have been found to be implicated in PD pathogenesis (MacMahon Copas et al., 2021). Surprisingly, most of these genes are highly expressed in astrocytes and microglia compared to neurons, thus providing further evidence that neurodegeneration is not a neuron-autonomous process but instead results from a complex crosstalk between a multiplicity of cell types within the brain. Several studies in rodents have evaluated the role of *PARK7* (Goldberg et al., 2005; Rousseaux et al., 2012), *PARK2, PINK1, SNCA* (Abeliovich et al., 2000; Burré et al., 2010; Cabin et al., 2005; Cooper et al., 2006), and *LRRK2* and have described astrocytes' (Barodia et al., 2019; Choi et al., 2016, 2019; Gu et al., 2010; Lee et al., 2019) and microglial' (Dionísio et al., 2019; Duffy et al., 2018; Dwyer et al., 2020; Nash et al., 2017; Sun et al., 2018) functions to be critically impaired and constitute the first dysfunctions that then may be fueling other PD features, ultimately leading to neuronal loss. Interestingly, these features have also been observed using hiPSC-derived *in vitro* systems (Aflaki et al., 2020; Booth et al., 2019; Panagiotakopoulou et al., 2020; Sonninen et al., 2020), corroborating the findings observed in human models. Notwithstanding the key contributions of animal models, it is imperative to note that the vast majority of PD cases are sporadic and only rare familial cases are due to specific genetic defects. Even more problematic is the fact that no animal model recapitulates the full spectrum of PD features (Dawson et al., 2010). For example, *Lrrk2*, *Snca*, *Parkin*, and *Pink1* transgenic mice do not exhibit substantial neurodegeneration (Daher et al., 2009; Kitada et al., 2009; Li et al., 2009), although, when PARKIN and PINK1 are deleted in adulthood, there is DA neurons degeneration (Lee et al., 2017; Shin et al., 2011). These conspicuous limitations of PD animal models necessitated the use of human cellular models to examine the PD genes' impact in a human background or the use of xenografting

approaches that would enable probing the links between PD genes and neurodegeneration.

The use of *in vitro* disease modeling strategies, mainly patient-derived iPSCs differentiated into DA neurons (Chambers et al., 2009; Doi et al., 2014; Kirkeby et al., 2012; Kriks et al., 2011; Mahajani et al., 2019; Schweitzer et al., 2020; Song et al., 2020), has dramatically advanced our understanding of PD initiation and progression and has facilitated the discovery of novel therapeutic solutions in relevant human cellular models. For instance, Ryan and colleagues used small-molecule high-throughput screening of a library of compounds to reveal the ability of isoxazole to specifically target *MEFC2-PCG1α* pathway, preventing neuronal damage associated with PD (Ryan et al., 2013). Naturally, potential targets which are identified through screenings may be validated using iPSC-derived neurons. For example, Soldner et al. validated an *SNCA* variant, in a non-coding distal enhancer element that regulates the expression of *SNCA*, which is associated with PD with use of CRISPR-Cas9-edited iPSC-derived DA neurons (Soldner et al., 2016). More recently, iPSC-based models revealed that in young-onset PD reduced function of lysosomes underlies α-synuclein accumulation and phosphorylation of protein kinase C (PKC). Phorbol esters, which control PKC activity and enhance lysosomal function, rescued the disease phenotype (Laperle et al., 2020), uncovering a novel candidate for therapeutic intervention.

The recent advances in 3D brain organoid technology promises to further improve our understanding of human-specific features linked with development, disease progression, and unique phenotypic profiling of genetic/sporadic PD. *In vitro* complex systems like midbrain organoids have put forward a new platform for uncovering the key factors that mediate PD pathological phenotypes, as well as for testing new compounds. Several research groups have indeed started to assess the contribution of these models and have shown that brain and midbrain-like 3D organoids recapitulate disease phenotypes and can elucidate unexplored aspects of this neurodegenerative disorder (Jo et al., 2016; Monzel et al., 2017; Smits et al., 2019; Wulansari et al., 2021). A clear example is the discovery of new direct functional connections that interfere with α-synuclein turnover and could not be captured in any human model besides 3D organoid cultures (Kim et al., 2019). While it has been reported that Thioredoxin-interacting protein (TXNIP) is linked with α-synuclein-induced PD (Su et al., 2017), Kim and colleagues have described a previously unknown interaction that places leucine-rich repeat kinase 2 (LRRK2) upstream of TXNIP in the regulation of α-synuclein and PD development (Kim et al., 2019).

Notwithstanding the remaining challenges, including a more faithful recapitulation of age-related changes, neurodegenerative diseases such as PD and AD present a fertile ground for unleashing the potential of cell-replacement therapies and have generally been a success story. Although the first grafting experiments with fetal DA neurons in PD were performed already in the 1980s (Brundin et al., 1986; Dunnett et al., 1981; reviewed in Barker et al., 2015; Steinbeck et al., 2015), it was the advent of cell reprogramming and advancements in neuronal differentiation protocols (Chambers et al., 2009) that led more recently to an "explosion" of xenografting studies (Doi et al., 2014; Gantner et al., 2020; Grealish et al., 2014; Kikuchi et al., 2017; Kirkeby et al., 2012; Kriks et al., 2011; Song et al., 2020; Steinbeck et al., 2015; Xiong et al., 2021). All these studies were transformative for the field mainly because of 3 key observations. First, they showed that iPSC can provide an unlimited source for DA neurons, without the technical and ethical complications of fetal tissue. Second, they provided compelling evidence for efficient generation of mesencephalic DA neurons and enhanced our understanding of cellular and molecular mechanisms of midbrain DA neurons differentiation and integration into a disease-relevant physiological environment. Third, they allowed us to assess *in vivo* the suitability of cell-replacement therapy in disease-relevant behaviors as it demonstrated that grafting of iPSC-derived DA neurons reversed PD symptoms in animal models, paving the way for large-scale clinical trials. All these were recently exemplified by Studer and Tabar labs, which developed a novel strategy to differentiate hPSCs into midbrain DA neurons based on a two-step wingless-related integration site (WNT) signaling activation to increase scalability and purity of midbrain DA neurons and reach clinical-scale production suitable for transplantation (Kim et al., 2021). These efforts brought hESC-derived DA neurons closer to clinical trials, by providing a large-scale, cryopreserved DA neuron progenitor product with an excellent toxicology profile and biodistribution readily available for clinical studies (Piao et al., 2021).

Epilepsy

One of the most common and disabling neurological disorders affecting all age groups, epilepsy is the result of imbalances between excitation and inhibition causing abnormal excessive or synchronous neuronal activity in the brain (Jefferys, 1994; Matsumoto and Marsan, 1964; McCormick and Contreras, 2001; Stafstrom and Carmant, 2015). The International League Against Epilepsy (ILAE) defines epilepsy as a group of disorders of the brain characterized by the periodic and unpredictable occurrence of seizures (Berg et al., 2010; Scheffer et al., 2017). Among many different types, temporal lobe epilepsy (TLE) with hippocampal sclerosis is one of the most prevalent forms of focal epilepsy. Data from patients indicate that this condition starts in adolescence and is often associated with an initial precipitating event during early childhood, such as febrile

seizures, encephalitis, or brain trauma (Berg et al., 1999; Hauser and Kurland, 1975; Hauser et al., 1993). On the other hand, studies using brain tissue from patients with drug-resistant focal epilepsy have shown that the most common pathologic hallmark is hippocampal sclerosis characterized by segmental loss of principal pyramidal neurons, synaptic reorganization, and reactive astrogliosis (Blumcke et al., 2017; Cavanagh and Meyer, 1956; Kim et al., 1990; de Lanerolle et al., 1989; Margerison and Corsellis, 1966; Sommer, 1880). Nevertheless, *ex vivo* approaches in humans cannot provide evidence on the origin of pathological features, namely, if hippocampal sclerosis and neuronal loss are caused by seizures alone or if the primary *noxa* is the triggering event and seizures occur as a consequence of that, further exacerbating brain damage. Therefore, the need for alternative experimental models was and still is pressing.

Animal models have made, by far, the biggest contribution toward exploring the fundamental mechanisms of the epileptogenic process, seizure generation, evolution of brain damage, and discovery of antiepileptic drugs. Starting in the late thirties, the first animal model of epilepsy was used for testing the efficacy of anti-seizure compounds (Putnam and Merritt, 1937). This was later followed by additional animal models, in which the seizures were induced either chemically or electrically and spontaneous genetic models (Ben-Ari et al., 1979; Cavalheiro et al., 1991; Goddard et al., 1969; Green and Sidman, 1962; Richards and Everett, 1946; Swinyard, 1949; Toman et al., 1946; Vergnes et al., 1982). Progress in gene-editing techniques led to the generation of induced genetic mouse models that have been important for understanding the molecular basis of neuronal circuit deficits and advancement of therapeutic solutions (Jones and Baraban, 2007; Wang et al., 2007; Yu et al., 2006). An example from our own work illustrates the utility of animal models for understanding aspects of epilepsy pathophysiology that are not possible in postmortem or in clinical setting. For many years, epileptologists argued over the effect of seizure activity on the development of brain damage (de Curtis et al., 2021). While some studies show that this is in fact true for some types of convulsive *status epilepticus* (SE) (Gorter et al., 2001; Pitkänen et al., 2002; Schwob et al., 1980), recent evidence from animal studies has disputed this notion and shows that in non-convulsive SE this is not the case (Arabadzisz et al., 2005; Noè et al., 2019; Riban et al., 2002; Vila Verde et al., 2021). Recently, Vila Verde and colleagues showed that, in animal models of non-convulsive SE triggered by the injection of chemiconvulsant kainic acid into the hippocampus, the secondary spread of seizure activity to regions distant from the injection site does not cause detrimental changes in the brain. However, when combined with a coexisting insult, seizures can work synergistically to further exacerbate the damage done by the underlying focal SE cause (Noè et al., 2019; Vila Verde et al., 2021).

Although epilepsy animal models have high predictive validity and have made crucial contributions to drug discovery and understanding of disease mechanism, the species-specific differences between humans and animals in disease pathophysiology, including even the seizure phenotype, remain (Bertram, 2007; Schauwecker, 2011).

Human-derived iPSCs and their neuronal derivatives have been increasingly utilized to investigate mostly genetic forms of epilepsy, including multisystemic neurodevelopmental disorders with a strong epilepsy phenotype such as Dravet syndrome (Kim et al., 2018; Liu et al., 2016; Sun et al., 2016), tuberous sclerosis (Blair et al., 2018; Winden et al., 2019; Zucco et al., 2018), Rett syndrome (Marchetto et al., 2010; Samarasinghe et al., 2021; Tang et al., 2016), and other rare epilepsy syndromes (Bershteyn et al., 2017; Negraes et al., 2021; Steinberg et al., 2021; Uchida et al., 2017). Early studies with human *in vitro* iPSC models validated some of the neuronal features initially discovered using animal models in a strictly human background, namely altered neuronal morphology including soma size, neurite outgrowth, synapse formation, dendritic spine length, altered spontaneous activity, and ion current density (Higurashi et al., 2013; Jiao et al., 2013; Liu et al., 2013), thereby greatly increasing the translational relevance of the findings. Dravet syndrome is an early-onset refractory and devastating type of epilepsy typically caused by *de novo* heterozygous variants in *SCN1A* gene. Liu and colleagues differentiated patient-derived iPSCs into a mixed culture of pyramidal-shaped glutamatergic and bipolar-shaped GABAergic neurons and detected higher sodium currents and hyperexcitability in both neuronal subtypes (Liu et al., 2013), whereas others, depending on the specific *SCN1A* mutation, have observed deficits only in inhibitory neurons (Kim et al., 2018; Liu et al., 2016; Sun et al., 2016), as also seen in mouse models (Cheah et al., 2012; Rubinstein et al., 2015; Yu et al., 2006).

Using 3D brain organoid models Blair and colleagues addressed tuberous sclerosis disease variability and the formation of cortical dysplasias. In a series of elegant experiments employing CRISPR-Cas9 techniques to generate constitutive and conditional knockout (KO) cell lines, they provided evidence for the "second-hit" hypothesis of cortical tuber formation and implicated specific cell population in disease initiation (Blair et al., 2018). Brain organoids were also utilized to examine neural oscillations in Rett syndrome. Specifically, by combining two-photon calcium imaging, extracellular recordings, and single-cell transcriptomic profiling in fused dorsal-ventral forebrain organoids, the authors revealed epileptiform-like activity and neuronal hyperexcitability in patients' organoids,

phenotypes that could be rescued by treatment with a p53 inhibitor (Samarasinghe et al., 2021).

As seen from the aforementioned examples, *in vitro* human cellular models have contributed tremendously to our understanding of the pathophysiological basis underlying human epilepsies and have accelerated the development of therapeutic drugs. Notwithstanding, iPSC-derived models suffer from elevated variability, inconsistent cellular composition, lack of non-neuronal cells (e.g., microglia), lack of vascularization, and inaccessibility of behavioral readouts. Some of these limitations could be overcome with xenografting models.

Human *in vitro* models, coupled with *in vivo* animal models, advance epilepsy research one step closer to finding therapeutic approaches that better fit the needs of specific patients with different genetic backgrounds. In addition to antiepileptic drugs and brain surgeries, stem cell therapy is a viable option for the treatment of various forms of epilepsy. Recent compelling evidence supporting the therapeutic use of this method has been published, and preclinical and clinical studies validated the use of disease-relevant cell types, including hippocampal precursor cells, neural stem cells, and GABAergic and mesenchymal cells, in ameliorating GABA-deficient firing pathways and restoring neuronal circuitry function (Chu et al., 2004; Cunningham et al., 2014; Lee et al., 2014; Upadhya et al., 2016; Waldau et al., 2010; Waloschková et al., 2021; Xu et al., 2019a; Zhu et al., 2023). In particular, Upadhya and colleagues tested the potential of MGE-like interneuron precursors derived from hiPSCs in alleviating TLE symptoms. Through the use of video-electroencephalographic recordings and behavioral tests in a rat model, they showed that xenografting of MGE-like precursors into the rat hippocampus after SE greatly reduced spontaneous recurrent seizures and improved cognitive, memory, and mood impairments (Upadhya et al., 2019).

Likewise, interneurons derived from hPSC and grafted into the brain of mice migrated extensively in order to integrate with the host circuitry and remarkably, even before fully maturing electrophysiologically, were able to suppress seizures and associated cognitive and behavioral deficits (Cunningham et al., 2014). Other reports highlighted the capability of GABAergic neurons derived from neural stem cells in reducing the frequency and duration of spontaneous recurrent seizures in chronically epileptic rats following their grafting into hippocampi (Xu et al., 2019a). Very recently, grafted human-derived GABAergic cortical interneurons in mouse models exhibited long-term efficacy in aborting seizures and ameliorating cognitive deficits without the risk of over-inhibition regardless of grafted neurons densities (Zhu et al., 2023). Although compelling evidence supports the use of human stem cells therapy for TLE and other forms of epilepsy, there are studies that have shown contradictory results. For instance, Anderson and colleagues discovered that, even though grafted human cells integrate, mature, and differentiate into GABAergic interneurons and become electrophysiologically active with mature firing patterns in the hippocampus, they do not suppress seizures (Anderson et al., 2018).

Transplantation approaches of interneurons in epilepsy seem to offer one of the most viable pathways for the development of novel therapeutic solutions, especially in drug-resistant patients. By reducing abnormal hyperexcitability and most importantly the recurrent seizures, interneuron cell-replacement therapy offers a better alternative to invasive surgery and the associated side effects, thereby dramatically improving quality of life and success rate (Backofen-Wehrhahn et al., 2018; Cunningham et al., 2014; Upadhya et al., 2019; Zhu et al., 2023). Nevertheless, major challenges remain, including the risk of tumor formation, altering brain excitation-inhibition balance, and cell-type heterogeneity of the graft (Amariglio et al., 2009; Anderson et al., 2018; Roy et al., 2006; Zhu et al., 2023).

Down syndrome

Down syndrome (DS) is the most common genetic cause of intellectual disability (ID), in which delayed and aberrant brain development lead to varying degrees of neurological and cognitive impairments (Down, 1995; Wilkins and Brody, 1971). With a frequency of approximately 1 in 800 births, DS occurs in all populations and is caused by a partial or complete trisomy of chromosome 21 (HSA21) (de Graaf et al., 2017; Hattori et al., 2000; Lejeune et al., 1959). Because gene expression is altered in all cells of the body, individuals with DS manifest a plethora of phenotypes including facial features, hearing and vision abnormalities, and cardiac and gastric malformations and have a higher propensity for comorbid conditions, such as hypothyroidism, autoimmune disorders, epilepsy, and dementia (Dahle and McCollister, 1986; Freeman et al., 1998; McCarron et al., 2014; Pueschel et al., 1991; Purdy et al., 2014; Roizen et al., 1994). Despite the heterogeneity in disease severity and the neurological phenotypes, some of the most common features observed in postmortem and clinical studies are the reduced brain volume accompanied with a smoother gyral appearance and condensed cortical surface area (Ferrer and Gullotta, 1990; Kesslak et al., 1994; Marin-Padilla, 1976; Patkee et al., 2020; Raz et al., 1995; Wisniewski, 1990).

As with other neurodevelopmental disorders, animal models have been instrumental in advancing our understanding of DS pathophysiology, in spite of the marked differences in genetics and developmental trajectories between humans and animals. A key moment in the field of DS was the advent of the Ts65Dn mouse model, which

harbors a segmental trisomy of a portion of mouse chromosome 16 with a syntenic region to HSA21 (Davisson et al., 1990; Reeves et al., 1995). This mouse model was transformative for DS research as the simultaneous overexpression of so many genes presented a better model compared to the laborious overexpression of single genes that was previously prevalent. Crucially, several phenotypic features seen in DS patients are recapitulated to different extents in the Ts65Dn mouse. For instance, Ts65Dn mice exhibit locomotor hyperactivity and have deficits in tasks that require integration of memory and visuospatial information and show diminished cognitive flexibility that worsens with age (Olmos-Serrano et al., 2016; Reeves et al., 1995). Furthermore, Ts65Dn mouse model exhibits adult-onset neurodegeneration (Granholm et al., 2000; Holtzman et al., 1996) and increased production of APP that is processed into soluble $A\beta_{40-42}$ (Hunter et al., 2003; Seo and Isacson, 2005). Ts65Dn mouse model has contributed to uncovering the roles and functions of specific HSA21 genes, including BRWD1, which is implicated in hippocampal long-term potentiation (LTP) and memory (Fulton et al., 2022), and OLIG1,2, the overexpression of which causes neurogenesis defects and imbalances in neuronal excitation/inhibition balance (Chakrabarti et al., 2010). Over-inhibition is assumed to be an underlying mechanism of intellectual disabilities associated with DS, and the Ts65Dn mouse model served as a platform for testing the efficacy of GABAA antagonists in reversing cognitive impairments and neurophysiological defects (Fernandez et al., 2007). Progress in genome engineering enabled the development of more DS mouse models that also recapitulate to varying degrees aspects of DS seen in humans (Li et al., 2007; Sago et al., 1998; Yu et al., 2010). Although mouse models made possible major advancements in DS research, the differences in genetics between mouse and human are insurmountable and this discordance limits the suitability of animal models. Consequently, there is the need for human experimental models that contain the exact DS genomic rearrangements.

Evidence suggests that the brain of children with DS develops differently (Baburamani et al., 2020; Contestabile et al., 2007; Guidi et al., 2008; Larsen et al., 2008; Stagni et al., 2020; Suetsugu and Mehraein, 1980; Takashima et al., 1981), and hiPSC-based *in vitro* models are an ideal tool for studying neurodevelopment. Studies using neurons from DS patients have revealed alterations in synaptic activity and density, axonal transport and synaptic vesicle cycling, and increased levels of Aβ and tau phosphorylation and have uncovered the roles of HSA21 genes such as *APP, DYRK1A*, and *BACE2* in these processes (Alić et al., 2021; Lu et al., 2013; Ovchinnikov et al., 2018; Shi et al., 2012; Weick et al., 2013; Wu et al., 2022). Interestingly, neurodevelopmental disease-relevant molecular alterations could be evident even in iPSC stage as we showed (Adamo et al., 2015) or in unrelated cellular populations such as fibroblasts. To study the protein landscape and turnover due to trisomy, Liu and colleagues compared DS fibroblasts with those from controls using sequential window acquisition of all theoretical fragment ion spectra (SWATH) mass spectrometry and revealed a DS-specific phenotype related to mitochondrial protein downregulation (Liu et al., 2017). Strikingly, the mitochondrial defects phenotype was recently replicated in DS patient-derived GABAergic neuronal cultures and MGE organoids (Xu et al., 2022). Instead, Meharena and colleagues targeted iPSC-derived neural progenitors from DS patients to reveal global changes related to T21. Specifically, they combined high-throughput chromosome conformation capture (Hi-C), assay for transposase-accessible chromatin using sequencing (ATAC-seq), and transcriptomic profiling to reveal global loss of chromatin accessibility, long-range chromatin interactions, and oxidative stress consistent with senescent cells. Interestingly, treatment with a cocktail of senolytic drugs ameliorated deficits in transcriptional regulation, proliferation, and cellular migration (Meharena et al., 2022).

The use of 3D brain organoids models is becoming increasingly relevant for the study of neurodevelopment generally and DS specifically, mainly because they recapitulate salient aspects of brain development (Kelley and Paşca, 2022). For instance, the use of iPSC-derived cerebral organoids from patients enabled Tang and colleagues to reveal differences in the shape and size of DS organoids probably due to impairments in neurogenesis as shown by diminished proliferation and decreased expression of layer II and IV markers in cortical neurons, underscoring cerebral organoids as valuable *in vitro* models (Tang et al., 2021). Besides having a reduced number of neurons, it has been documented using human studies that DS brains have nearly twice as many astrocytes compared with age-matched controls (Griffin et al., 1998; Mito and Becker, 1993; Zdaniuk et al., 2011). These deficits in cell lineage ratio are likely to cause impairments in dendritic arborization, synaptogenesis, and synaptic plasticity, since astrocytes modulate and sustain these vital neuronal functions through the expression and/or release of various neuroactive molecules. Recent studies have identified astrocytes as potential targets to alleviate DS symptoms as different labs have reported that iPSC-derived astrocytes i) are critical for controlling synaptogenesis and mTOR pathway (Araujo et al., 2018), ii) display genome-wide perturbations in gene expression, an altered cell adhesion profile, and increased cellular motility and dynamics (Ponroy Bally et al., 2020), and iii) regulate aberrant astrocyte proliferation observed in DS (Kawatani et al., 2021).

To harness the potential of xenografting approaches and validate the key role of astrocytes in DS pathophysiology,

iPSC-derived astrocytes were grafted into the lateral ventricles of neonatal mice brains (Chen et al., 2014). Grafted astrocytes integrated into the host neuronal circuits and critically, through the release of neuroactive molecules, modulated endogenous neurogenesis and cell proliferation. Likewise, Real and colleagues grafted patient iPSC-derived neurons and used single-cell-resolution intravital microscopy to gain insights into the dynamics of neuronal pruning, synaptogenesis, and network activity during the earliest stages of cortical development. Grafted cortical excitatory neurons from both control and DS groups exhibited axonal outgrowth, dendrite pruning, and functional connections with host neurons. However, only DS-derived neurons demonstrated higher dendritic spine stability marked by a decrease in dendritic spine turnover, which led to a reduction in network activity (Real et al., 2018). To elucidate the mechanisms underlying the GABAergic dysfunction observed in DS, Huo and colleagues observed cellular and migration deficits and a reduction in the number of cortical interneurons. The interneuron migration deficit was present also following the grafting of DS interneurons in the medial septum of mice, suggesting that the reduced cortical interneuron number may be a result of deficits in migration (Huo et al., 2018). In contrast, following grafting of DS patients-derived brain organoids into the mouse brain, Xu et al. demonstrated that upregulated expression of *OLIG2* in DS neural progenitors causes overproduction of subclass-specific GABAergic interneurons and that reduction of *OLIG2* expression improved recognition memory and interneuron differentiation (Xu et al., 2019c). This contradiction regarding the number of GABAergic interneurons in DS could have been resolved by quantifying specific populations of interneuron subtype (e.g., calretinin+, calbindin+), as HSA21 genes could differentially affect the production of specific interneuron subtypes. As shown earlier, DS has been associated with cellular senescence (Meharena et al., 2022). Jin and colleagues combined microglia-containing brain organoids and mouse chimeras to show that senescence also affects non-neuronal microglia. Specifically, following the derivation of microglia from DS iPSC, they dissected microglia function in microglia-containing organoids following microglia grafting in the neonatal mouse brain. Microglia exhibited an enhanced synaptic pruning, which in turn affected the synaptic neurotransmission. Following exposure to human-derived tau, engrafted microglia underwent cellular senescence, which was rescued upon the specific downregulation of HSA21 gene *IFNAR* (Jin et al., 2022).

As with other brain diseases, 2D and 3D patient-derived *in vitro* models made it possible to identify DS phenotypes specifically due to the chromosomal abnormality and the affected genes, which was not possible previously in animal models, also because animal and human genomes do not always align. As a matter of fact, Ts65Dn gold standard mouse model contains an additional 45 protein-coding genes that are not present in patients. Critically, those genes are important for neurodevelopment (Guedj et al., 2023), which questions the validity of the model and might explain why promising treatments in the mouse were met with disappointment in clinical trials (https://clinicaltrials.gov/, reviewed in Lee et al., 2020). In contrast, the integration of animal models with human *in vitro* models often leads to a more complete understanding of disease pathophysiology because it enables the interrogation and validation of DS-specific cellular and molecular phenotypes in their physiological milieu and their impact on behavioral readouts, respectively. Hereby, xenografting approaches combine the fidelity of patient-specific cellular models that leads to a better understanding of disease pathophysiology with an experimental platform that accelerates the identification, screening, and efficacy assessment of novel drug compounds.

Schizophrenia

The general consensus indicates that schizophrenia (SCZ) is a neurodevelopmental disorder that manifests in late adolescence, but a prodromal phase is already evident in childhood. A prevalent brain disorder, SCZ, features episodes of psychosis, hallucinations, and disorganized thinking (positive symptoms), invariably coupled to blunted affect, anhedonia, and asociality (negative symptoms) (Bleuler, 1950; Hulshoff Pol et al., 2000; Murray and Lewis, 1988; St Clair et al., 2005; Susser and Lin, 1992).

Most of our neurobiological insights in SCZ are the results of decades of research in animal models. Rodent models have been instrumental for increasing our understanding of genetics contribution to SCZ, environmental risk factors, and brain and behavioral abnormalities associated with SCZ in humans (Abazyan et al., 2010; Brody et al., 2004; Geyer et al., 1993; Liang et al., 2022; Ma et al., 2013; Mätlik et al., 2022; Moghaddam et al., 1997). An illustrative example that emphasizes the advantages of xenografting approaches over animal models is the dopamine hyperactivity in the mesolimbic system hypothesis of SCZ (Toda and Abi-Dargham, 2007). Work in rodent models has revealed that loss of GABAergic activity in ventral hippocampus drives the increased dopamine signaling in the mesolimbic system and that restoration of aberrant hippocampal activity with pharmacological manipulation or deep brain stimulation reverses SCZ-like phenotypes in rodent models (Boley et al., 2014; Lodge and Grace, 2007; Perez et al., 2013). Despite the key contributions derived from animal models, a direct link between the putative reduced GABAergic activity in the hippocampus triggering aberrant dopamine signaling and SCZ-relevant behavioral

alterations was lacking. Consequently, the grafting of mouse ESC or MGE-derived rat's interneurons into the hippocampus of a rat SCZ model restored both aberrant GABAergic and DA activity in hippocampus and ventral tegmental area, respectively. Importantly, the grafts rescued deficits in social interaction and cognitive flexibility underscoring the edge of graft-based models in modeling complex neuropsychiatric disorders (Donegan et al., 2017; Perez and Lodge, 2013).

In the last decade, human cell-based *in vitro* models—owing to their human genetic background—have elucidated important aspects of SCZ cellular and molecular underpinnings that so far were not possible in animal models. Patients' fibroblasts were reprogrammed into iPSC and then differentiated into neurons or assembled into brain organoids. A comprehensive analysis of neurons revealed reductions in neuronal connectivity, neurite outgrowth, and synaptic proteins along with altered gene expression related to WNT signaling, mitochondrial function, and excitatory/inhibitory balance (Brennand et al., 2011; Kathuria et al., 2020; Ni et al., 2020). Remarkably, many of the disease phenotypes were amenable to treatment with common antipsychotic medication (Brennand et al., 2011), highlighting the translational potential of cellular models. Likewise, patient-derived iPSC models were successfully employed to study even predisposition to cellular defects associated with SCZ. In particular, transcriptomic analysis of neural progenitors derived from patient iPSC uncovered altered gene expression in cytoskeletal remodeling and oxidative stress, predictive of defects in progenitors' migration and cellular metabolism, respectively. As predicted, neural progenitors exhibited defects in migration and elevated oxidative stress (Brennand et al., 2015). The advent of CRISPR-Cas9 gene-editing technologies enabled an elegant dissection of the contribution of SCZ common variants and top candidate genes on neuronal function. The use of CRISPRa/i to upregulate or repress the endogenous expression of top SCZ genes in patient-derived neurons revealed specific effects on pre- and postsynaptic neuronal domains and genotype-specific transcriptional alterations, while combinatorial gene perturbations uncovered convergence on synaptic physiology and synergies between SCZ risk variants (Schrode et al., 2019).

Microglia have emerged as a critical population of CNS cells with important roles in SCZ pathophysiology. Their contribution was highlighted using patient-derived neuronal cultures. Human monocyte-derived microglia-like cells promoted excessive synaptic pruning when co-cultured with SCZ patient-derived neurons, reminiscent of reduced synaptic density found in postmortem SCZ brain tissue (Sellgren et al., 2019). Intriguingly, treatment with antibiotic minocycline normalized microglia-induced synapse elimination and was associated with a decreased SCZ incidence in high-risk individuals, suggesting that targeting synaptic pruning could be a viable treatment option for SCZ (Sellgren et al., 2019). More recently, activated microglia co-cultured with SCZ patient-derived inhibitory interneurons—a neuronal population affected in SCZ—caused a range of metabolic deficits in the latter, including mitochondrial dysfunction and further impairments in neuronal arborization, synaptogenesis, and GABA release. In contrast to interneurons from control individuals, the metabolic deficits persisted in interneurons from SCZ patients long after the removal of the inflammatory stimulus, providing evidence for synergies between SCZ genetic predisposition and environmental risk factors (Park et al., 2020). Although the aforementioned studies provided strong evidence for the key role of neuroglia in SCZ-associated pathology, demonstrating a causative role between neuroglia defects and SCZ onset requires validation in disease-relevant *in vivo* models. In that regard, iPSCs from children with SCZ were differentiated into glia progenitor cells *in vitro* and subsequently xenografted into neonatal mice. In addition to deficits in glial cortical migration, astrocytic maturation, and hypomyelination, grafted mice exhibited excessive anxiety, sociality defects, and sleep disturbances, providing direct evidence for a causative role between glial maturation and development of SCZ (Windrem et al., 2017).

As with other complex brain disorders, grafting approaches in SCZ bridged the gap between cellular mechanisms that were initially revealed in iPSC cellular models and their specific impact on disease-relevant behavioral alterations, which is possible only in animal models. Furthermore, grafting approaches in SCZ provided an experimental platform to test disease-relevant experimental hypotheses (Perez and Lodge, 2013) and uncovered a key role for neuroglia in the onset of SCZ (Windrem et al., 2017). All these major advancements would not have been possible neither with animal models nor with cellular models alone. Notably, iPSC-based neuronal cultures lack behavioral readouts, which makes it impossible to causatively link cellular phenotypes with the disease, while animal models have limited construct and face validity.

INTEGRATING XENOGRAFTING APPROACH WITH TECHNOLOGICAL ADVANCEMENTS

Finally, building on the enabling edges recounted earlier in bridging disease modeling across the *in vitro*/*in vivo* gap, xenografting can now be envisioned as a unique experimental platform to integrate recent technological breakthroughs spanning methods that enable cellular, molecular, and functional analysis; drug development; and personalized medicine into the disease modeling pipeline of the brain and its pathologies (Figure 3). In that regard,

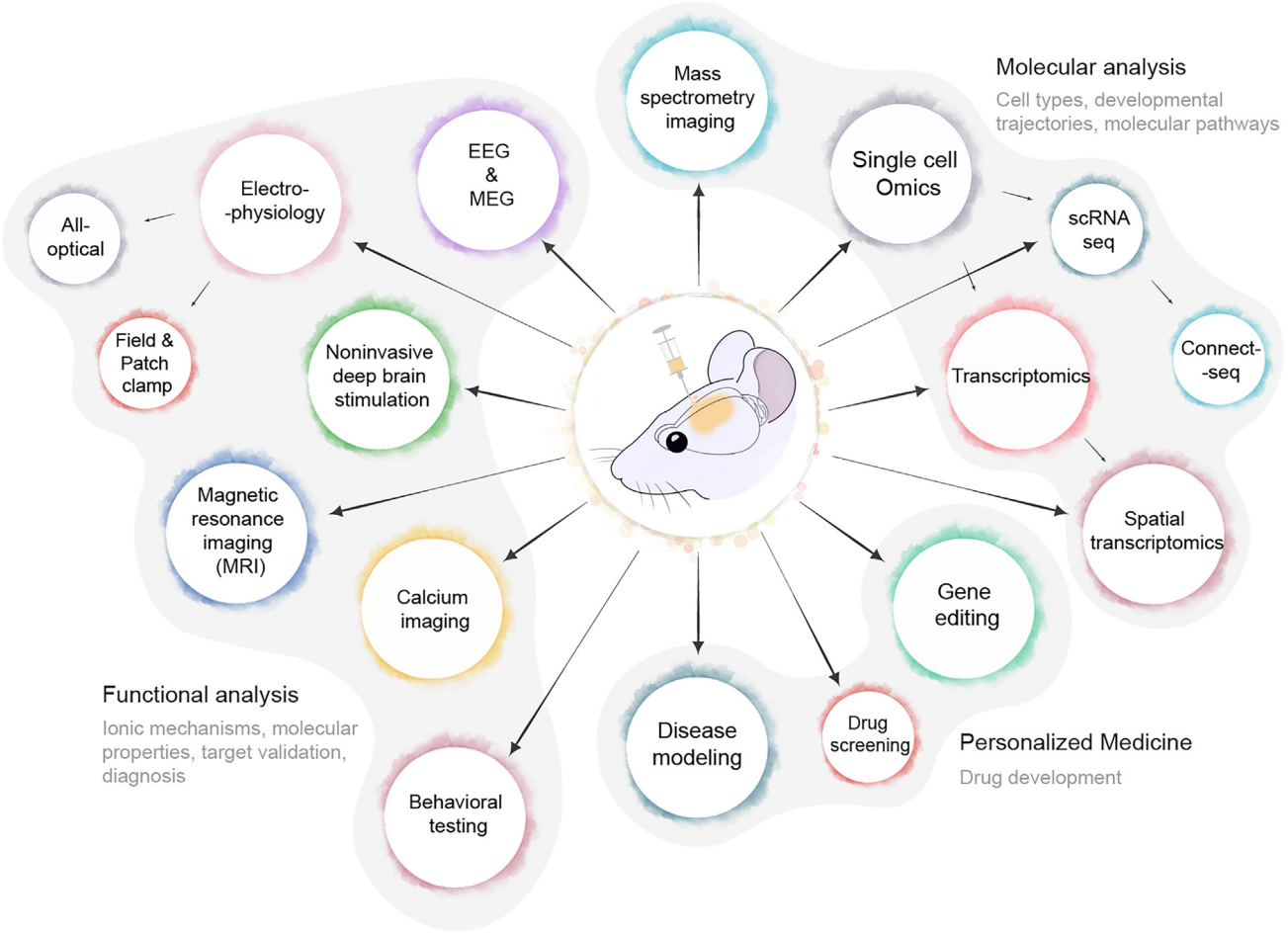

Figure 3. Integrating xenografting with methodological and technological advancements

Owing to its versatility, xenografting can be integrated with cutting-edge technologies enabling the molecular and functional analysis of brain disorders and accelerating the development of efficient medications for personalized medicine.

some prominent examples include the report of a proof-of-concept approach to target noninvasively with unprecedented precision deep brain tissue by application of electromagnetic fields of different frequencies (Grossman et al., 2017). The approach was validated in a behaving mouse by modulating the firing properties of deep hippocampal neurons, while sparing the overlying tissue. Besides its major implications for the treatment of neurological and neuropsychiatric diseases (Lozano, 2017), noninvasive neuromodulation opens new frontiers for unraveling neuronal circuit formation and function. We envisage a scenario in which, following engraftment of *in vitro*-generated 2D or 3D neuronal tissue, we would be able to target with high precision either the whole graft or parts of it, while sparing the surrounding tissue and elucidate how the graft integrates within the endogenous circuits and modulates behavior and function (Linaro et al., 2019) in real time. An alternative which enables cell-type specificity and unprecedented high throughput is the advent of all-optical electrophysiology. The combination of novel channelrhodopsin variants (CheRiff) with exquisite light sensitivity with rapid membrane voltage indicators (QuasAr) and near-infrared fluorescence mounted into a single co-expression vector (Optopatch) enables the simultaneous stimulation and recording of neurons (Hochbaum et al., 2014) without the need for the classical yet laborious and time-consuming patch-clamp electrophysiology. This powerful technique was validated *in vitro* by high-throughput functional characterization of iPSC-derived amyotrophic lateral sclerosis motor neurons (Kiskinis et al., 2018) and more recently *in vivo* for dissecting cortical layer 1 local neural circuits roles and functioning in sensory integration (Fan et al., 2020). Only now can we start integrating xenografting of healthy or diseased iPSC-derived neuronal models with all-optical electrophysiology for high-throughput tracing of graft maturation, circuit

formation and modulation, and their real-time impact on behavior and cognition.

Major advances in single-cell-omics approaches including proteome sequencing at single-amino-acid resolution (Alfaro et al., 2021; Brinkerhoff et al., 2021) have afforded an unprecedented view on cell diversity, heterogeneity, and function; however, these technologies lack spatial information. Recent developments in spatial transcriptomics and mass spectrometry imaging promise to overcome these drawbacks by uncovering the spatial information of mRNA, peptides, and metabolites (Caprioli, 2019; Rodriques et al., 2019; Ståhl et al., 2016), thus ushering in a comprehensive, global view of the molecular mechanisms in health and disease. Moreover, we have the opportunity to combine transcriptional profiling with neuronal connectomics such as in Connect-seq where neuronal viral tracing and single-cell RNA sequencing go hand in hand to reveal the molecules that establish and modulate neural circuitries formation and their functioning (Hanchate et al., 2020). All these technological advancements allow us to design experiments in which *in vitro*-generated neural models and their engraftment into the brain of animal models will enable us to examine the transcriptional and proteomic profile of grafted single neurons, visualize the location of this activity, and track how this activity establishes the neuronal connections that sustain behavior. Through these approaches, xenografting becomes thus the experimental conduit to translate the repertoire of *in vitro*-generated human neuronal identities (resolved at single-cell-omics detail) into *in vivo* functions (by selectively targeting and manipulating them *in vivo*) to gain insights about behavior, cognition, and neuronal plasticity.

CONCLUSION

A great deal of what we know about the brain and its function derives from the study of animal models, which has been and still is indispensable for insights on behavior, cognition, drug development, and disease pathophysiology. However, animal models are not human and despite many conserved cellular and molecular processes the species-specific differences have limited their use. On the other hand, *in vitro* neural models, owing to their reductionistic nature, have been instrumental for improving our understanding of molecular mechanisms of neuronal differentiation and cellular fate decisions. Notwithstanding its advantages, reductionism is a double-edged sword as it is not compatible with the complexity and the intricate functions of the brain. Furthermore, despite progress in neuronal media composition and neuronal differentiation protocols, *in vitro* neurons do not reach full maturation, lack key nutrients and signals provided by other brain cell types, are devoid of sensory input, and finally do not allow assessment of "face validity" which is key for neuropsychiatric disorders modeling (Pașca, 2024). In an attempt to harness the best of both worlds, scientists have xenografted *in vitro*-generated human neural models into the brains of animal models (e.g., rodents). Advantages of xenografting approaches can be categorized into 3 specific domains: i) optimization of human *in vitro* cellular models as they enable the advanced maturation of neurons *vis-à-vis in vitro* cultures, ii) provision of an advanced platform for drug testing by bridging the gap between the need for human models and the importance of assessing the impact of novel compounds on functioning neuronal circuits and behavioral readouts and disease mechanisms *in vivo*, and iii) becoming a stepping stone for the advancement of cell-replacement therapies toward clinical trials (Pașca, 2024). Here we provided a systematic account of few paradigmatic yet common neuropsychiatric and neurological disorders, and most importantly the juxtaposition of findings derived from purely *in vitro*, *in vivo*, and xenografting approaches clearly highlights the edge offered by the latter. The integration of xenografting approaches with unique cutting-edge technologies brings their utility to a new dimension and affords the optimism that interrogation of previously untenable aspects of brain physiology becomes now the benchmark in the field of modern human neurobiology.

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AUTHOR CONTRIBUTIONS

Conceptualization: M.F.P., R.S., and G.T. Writing: M.F.P., R.S., and G.T. Figures: M.F.P.

DECLARATION OF INTERESTS

The authors declare no competing interests.

REFERENCES

Abazyan, B., Nomura, J., Kannan, G., Ishizuka, K., Tamashiro, K.L., Nucifora, F., Pogorelov, V., Ladenheim, B., Yang, C., Krasnova, I.N., et al. (2010). Prenatal interaction of mutant DISC1 and immune activation produces adult psychopathology. Biol. Psychiatry *68*, 1172–1181. https://doi.org/10.1016/j.biopsych.2010.09.022.

Abeliovich, A., Schmitz, Y., Fariñas, I., Choi-Lundberg, D., Ho, W.H., Castillo, P.E., Shinsky, N., Verdugo, J.M., Armanini, M., Ryan, A., et al. (2000). Mice lacking alpha-synuclein display functional deficits in the nigrostriatal dopamine system. Neuron *25*, 239–252. https://doi.org/10.1016/s0896-6273(00)80886-7.

Adamo, A., Atashpaz, S., Germain, P.-L., Zanella, M., D'Agostino, G., Albertin, V., Chenoweth, J., Micale, L., Fusco, C., Unger, C., et al. (2015). 7q11.23 dosage-dependent dysregulation in human pluripotent stem cells affects transcriptional programs in disease-relevant lineages. Nat. Genet. *47*, 132–141. https://doi.org/10.1038/ng.3169.

Aflaki, E., Stubblefield, B.K., McGlinchey, R.P., McMahon, B., Ory, D.S., and Sidransky, E. (2020). A characterization of Gaucher iPS-derived astrocytes: Potential implications for Parkinson's disease. Neurobiol. Dis. *134*, 104647. https://doi.org/10.1016/j.nbd.2019.104647.

Alfaro, J.A., Bohländer, P., Dai, M., Filius, M., Howard, C.J., van Kooten, X.F., Ohayon, S., Pomorski, A., Schmid, S., Aksimentiev, A., et al. (2021). The emerging landscape of single-molecule protein sequencing technologies. Nat. Methods *18*, 604–617. https://doi.org/10.1038/s41592-021-01143-1.

Alić, I., Goh, P.A., Murray, A., Portelius, E., Gkanatsiou, E., Gough, G., Mok, K.Y., Koschut, D., Brunmeir, R., Yeap, Y.J., et al. (2021). Patient-specific Alzheimer-like pathology in trisomy 21 cerebral organoids reveals BACE2 as a gene dose-sensitive AD suppressor in human brain. Mol. Psychiatry *26*, 5766–5788. https://doi.org/10.1038/s41380-020-0806-5.

Alzheimer, A. (1910). Beiträge zur Kenntnis der Pathologischen Neuroglia und ihrer Beziehungen zu den Abbauvorgängen im Nervengewebe (Jena: G. Fischer).

Alzheimer, A., Stelzmann, R.A., Schnitzlein, H.N., and Murtagh, F.R. (1995). An English translation of Alzheimer's 1907 paper, "Uber eine eigenartige Erkankung der Hirnrinde.". Clin. Anat. *8*, 429–431. https://doi.org/10.1002/ca.980080612.

Amariglio, N., Hirshberg, A., Scheithauer, B.W., Cohen, Y., Loewenthal, R., Trakhtenbrot, L., Paz, N., Koren-Michowitz, M., Waldman, D., Leider-Trejo, L., et al. (2009). Donor-derived brain tumor following neural stem cell transplantation in an ataxia telangiectasia patient. PLoS Med. *6*, e1000029. https://doi.org/10.1371/journal.pmed.1000029.

Anderson, N.C., Van Zandt, M.A., Shrestha, S., Lawrence, D.B., Gupta, J., Chen, C.Y., Harrsch, F.A., Boyi, T., Dundes, C.E., Aaron, G., et al. (2018). Pluripotent stem cell-derived interneuron progenitors mature and restore memory deficits but do not suppress seizures in the epileptic mouse brain. Stem Cell Res. *33*, 83–94. https://doi.org/10.1016/j.scr.2018.10.007.

Arabadzisz, D., Antal, K., Parpan, F., Emri, Z., and Fritschy, J.-M. (2005). Epileptogenesis and chronic seizures in a mouse model of temporal lobe epilepsy are associated with distinct EEG patterns and selective neurochemical alterations in the contralateral hippocampus. Exp. Neurol. *194*, 76–90. https://doi.org/10.1016/j.expneurol.2005.01.029.

Araque, A., Parpura, V., Sanzgiri, R.P., and Haydon, P.G. (1999). Tripartite synapses: glia, the unacknowledged partner. Trends Neurosci. *22*, 208–215. https://doi.org/10.1016/s0166-2236(98)01349-6.

Araujo, B.H.S., Kaid, C., De Souza, J.S., Gomes da Silva, S., Goulart, E., Caires, L.C.J., Musso, C.M., Torres, L.B., Ferrasa, A., Herai, R., et al. (2018). Down Syndrome iPSC-Derived Astrocytes Impair Neuronal Synaptogenesis and the mTOR Pathway In Vitro. Mol. Neurobiol. *55*, 5962–5975. https://doi.org/10.1007/s12035-017-0818-6.

Baburamani, A.A., Vontell, R.T., Uus, A., Pietsch, M., Patkee, P.A., Wyatt-Ashmead, J., Chin-Smith, E.C., Supramaniam, V.G., Donald Tournier, J., Deprez, M., and Rutherford, M.A. (2020). Assessment of radial glia in the frontal lobe of fetuses with Down syndrome. Acta Neuropathol. Commun. *8*, 141. https://doi.org/10.1186/s40478-020-01015-3.

Backofen-Wehrhahn, B., Gey, L., Bröer, S., Petersen, B., Schiff, M., Handreck, A., Stanslowsky, N., Scharrenbroich, J., Weißing, M., Staege, S., et al. (2018). Anticonvulsant effects after grafting of rat, porcine, and human mesencephalic neural progenitor cells into the rat subthalamic nucleus. Exp. Neurol. *310*, 70–83. https://doi.org/10.1016/j.expneurol.2018.09.004.

Barker, R.A., Drouin-Ouellet, J., and Parmar, M. (2015). Cell-based therapies for Parkinson disease—past insights and future potential. Nat. Rev. Neurol. *11*, 492–503. https://doi.org/10.1038/nrneurol.2015.123.

Barodia, S.K., McMeekin, L.J., Creed, R.B., Quinones, E.K., Cowell, R.M., and Goldberg, M.S. (2019). PINK1 phosphorylates ubiquitin predominantly in astrocytes. NPJ Parkinsons Dis. *5*, 29. https://doi.org/10.1038/s41531-019-0101-9.

Bassil, R., Shields, K., Granger, K., Zein, I., Ng, S., and Chih, B. (2021). Improved modeling of human AD with an automated culturing platform for iPSC neurons, astrocytes and microglia. Nat. Commun. *12*, 5220. https://doi.org/10.1038/s41467-021-25344-6.

Ben-Ari, Y., Tremblay, E., Ottersen, O.P., and Naquet, R. (1979). Evidence suggesting secondary epileptogenic lesions after kainic acid: pretreatment with diazepam reduces distant but not local brain damage. Brain Res. *165*, 362–365. https://doi.org/10.1016/0006-8993(79)90571-7.

Berg, A.T., Shinnar, S., Levy, S.R., and Testa, F.M. (1999). Newly diagnosed epilepsy in children: presentation at diagnosis. Epilepsia *40*, 445–452. https://doi.org/10.1111/j.1528-1157.1999.tb00739.x.

Berg, A.T., Berkovic, S.F., Brodie, M.J., Buchhalter, J., Cross, J.H., van Emde Boas, W., Engel, J., French, J., Glauser, T.A., Mathern, G.W., et al. (2010). Revised terminology and concepts for organization of seizures and epilepsies: report of the ILAE Commission on Classification and Terminology, 2005-2009. Epilepsia *51*, 676–685. https://doi.org/10.1111/j.1528-1167.2010.02522.x.

Bernheimer, H., Birkmayer, W., Hornykiewicz, O., Jellinger, K., and Seitelberger, F. (1973). Brain dopamine and the syndromes of Parkinson and Huntington. Clinical, morphological and neurochemical correlations. J. Neurol. Sci. *20*, 415–455. https://doi.org/10.1016/0022-510x(73)90175-5.

Bershteyn, M., Nowakowski, T.J., Pollen, A.A., Di Lullo, E., Nene, A., Wynshaw-Boris, A., and Kriegstein, A.R. (2017). Human iPSC-Derived Cerebral Organoids Model Cellular Features of Lissencephaly and Reveal Prolonged Mitosis of Outer Radial Glia. Cell Stem Cell *20*, 435–449.e4. https://doi.org/10.1016/j.stem.2016.12.007.

Bertram, E. (2007). The Relevance of Kindling for Human Epilepsy. Epilepsia *48*, 65–74. https://doi.org/10.1111/j.1528-1167.2007.01068.x.

Bhaduri, A., Andrews, M.G., Mancia Leon, W., Jung, D., Shin, D., Allen, D., Jung, D., Schmunk, G., Haeussler, M., Salma, J., et al. (2020). Cell stress in cortical organoids impairs molecular subtype specification. Nature *578*, 142–148. https://doi.org/10.1038/s41586-020-1962-0.

Blair, J.D., Hockemeyer, D., and Bateup, H.S. (2018). Genetically engineered human cortical spheroid models of tuberous sclerosis. Nat. Med. *24*, 1568–1578. https://doi.org/10.1038/s41591-018-0139-y.

Bleuler, E. (1950). Dementia Praecox or the Group of Schizophrenias (Oxford, England: International Universities Press).

Blumcke, I., Spreafico, R., Haaker, G., Coras, R., Kobow, K., Bien, C.G., Pfäfflin, M., Elger, C., Widman, G., Schramm, J., et al. (2017). Histopathological Findings in Brain Tissue Obtained during Epilepsy Surgery. N. Engl. J. Med. *377*, 1648–1656. https://doi.org/10.1056/NEJMoa1703784.

Boley, A.M., Perez, S.M., and Lodge, D.J. (2014). A fundamental role for hippocampal parvalbumin in the dopamine hyperfunction associated with schizophrenia. Schizophr. Res. *157*, 238–243. https://doi.org/10.1016/j.schres.2014.05.005.

Booth, H.D.E., Wessely, F., Connor-Robson, N., Rinaldi, F., Vowles, J., Browne, C., Evetts, S.G., Hu, M.T., Cowley, S.A., Webber, C., and Wade-Martins, R. (2019). RNA sequencing reveals MMP2 and TGFB1 downregulation in LRRK2 G2019S Parkinson's iPSC-derived astrocytes. Neurobiol. Dis. *129*, 56–66. https://doi.org/10.1016/j.nbd.2019.05.006.

Braak, H., and Braak, E. (1991). Neuropathological stageing of Alzheimer-related changes. Acta Neuropathol. *82*, 239–259. https://doi.org/10.1007/BF00308809.

Brennand, K., Savas, J.N., Kim, Y., Tran, N., Simone, A., Hashimoto-Torii, K., Beaumont, K.G., Kim, H.J., Topol, A., Ladran, I., et al. (2015). Phenotypic differences in hiPSC NPCs derived from patients with schizophrenia. Mol. Psychiatry *20*, 361–368. https://doi.org/10.1038/mp.2014.22.

Brennand, K.J., Simone, A., Jou, J., Gelboin-Burkhart, C., Tran, N., Sangar, S., Li, Y., Mu, Y., Chen, G., Yu, D., et al. (2011). Modelling schizophrenia using human induced pluripotent stem cells. Nature *473*, 221–225. https://doi.org/10.1038/nature09915.

Brinkerhoff, H., Kang, A.S.W., Liu, J., Aksimentiev, A., and Dekker, C. (2021). Multiple rereads of single proteins at single–amino acid resolution using nanopores. Science *374*, 1509–1513. https://doi.org/10.1126/science.abl4381.

Brody, S.A., Dulawa, S.C., Conquet, F., and Geyer, M.A. (2004). Assessment of a prepulse inhibition deficit in a mutant mouse lacking mGlu5 receptors. Mol. Psychiatry *9*, 35–41. https://doi.org/10.1038/sj.mp.4001404.

Brundin, P., Nilsson, O.G., Strecker, R.E., Lindvall, O., Astedt, B., and Björklund, A. (1986). Behavioural effects of human fetal dopamine neurons grafted in a rat model of Parkinson's disease. Exp. Brain Res. *65*, 235–240. https://doi.org/10.1007/BF00243848.

Burré, J., Sharma, M., Tsetsenis, T., Buchman, V., Etherton, M.R., and Südhof, T.C. (2010). Alpha-synuclein promotes SNARE-complex assembly in vivo and in vitro. Science *329*, 1663–1667. https://doi.org/10.1126/science.1195227.

Cabin, D.E., Gispert-Sanchez, S., Murphy, D., Auburger, G., Myers, R.R., and Nussbaum, R.L. (2005). Exacerbated synucleinopathy in mice expressing A53T SNCA on a Snca null background. Neurobiol. Aging *26*, 25–35. https://doi.org/10.1016/j.neurobiolaging.2004.02.026.

Caprioli, R.M. (2019). Imaging Mass Spectrometry: A Perspective. J. Biomol. Tech. *30*, 7–11. https://doi.org/10.7171/jbt.19-3001-002.

Carlsson, A., Lindqvist, M., and Magnusson, T. (1957). 3,4-Dihydroxyphenylalanine and 5-hydroxytryptophan as reserpine antagonists. Nature *180*, 1200. https://doi.org/10.1038/1801200a0.

Cavalheiro, E.A., Leite, J.P., Bortolotto, Z.A., Turski, W.A., Ikonomidou, C., and Turski, L. (1991). Long-Term Effects of Pilocarpine in Rats: Structural Damage of the Brain Triggers Kindling and Spontaneous I Recurrent Seizures. Epilepsia *32*, 778–782. https://doi.org/10.1111/j.1528-1157.1991.tb05533.x.

Cavanagh, J.B., and Meyer, A. (1956). Aetiological aspects of Ammon's horn sclerosis associated with temporal lobe epilepsy. Br. Med. J. *2*, 1403–1407. https://doi.org/10.1136/bmj.2.5006.1403.

Cenci, M.A., and Crossman, A.R. (2018). Animal models of l-dopa-induced dyskinesia in Parkinson's disease. Mov. Disord. *33*, 889–899. https://doi.org/10.1002/mds.27337.

Chakrabarti, L., Best, T.K., Cramer, N.P., Carney, R.S.E., Isaac, J.T.R., Galdzicki, Z., and Haydar, T.F. (2010). Olig1 and Olig2 triplication causes developmental brain defects in Down syndrome. Nat. Neurosci. *13*, 927–934. https://doi.org/10.1038/nn.2600.

Chambers, S.M., Fasano, C.A., Papapetrou, E.P., Tomishima, M., Sadelain, M., and Studer, L. (2009). Highly efficient neural conversion of human ES and iPS cells by dual inhibition of SMAD signaling. Nat. Biotechnol. *27*, 275–280. https://doi.org/10.1038/nbt.1529.

Cheah, C.S., Yu, F.H., Westenbroek, R.E., Kalume, F.K., Oakley, J.C., Potter, G.B., Rubenstein, J.L., and Catterall, W.A. (2012). Specific deletion of NaV1.1 sodium channels in inhibitory interneurons causes seizures and premature death in a mouse model of Dravet syndrome. Proc. Natl. Acad. Sci. USA *109*, 14646–14651. https://doi.org/10.1073/pnas.1211591109.

Chen, C., Jiang, P., Xue, H., Peterson, S.E., Tran, H.T., McCann, A.E., Parast, M.M., Li, S., Pleasure, D.E., Laurent, L.C., et al. (2014). Role of astroglia in Down's syndrome revealed by patient-derived human-induced pluripotent stem cells. Nat. Commun. *5*, 4430. https://doi.org/10.1038/ncomms5430.

Choi, D.-J., An, J., Jou, I., Park, S.M., and Joe, E.-H. (2019). A Parkinson's disease gene, DJ-1, regulates anti-inflammatory roles of astrocytes through prostaglandin D2 synthase expression. Neurobiol. Dis. *127*, 482–491. https://doi.org/10.1016/j.nbd.2019.04.003.

Choi, I., Choi, D.-J., Yang, H., Woo, J.H., Chang, M.-Y., Kim, J.Y., Sun, W., Park, S.-M., Jou, I., Lee, S.-H., and Joe, E.H. (2016). PINK1 expression increases during brain development and stem cell differentiation, and affects the development of GFAP-positive astrocytes. Mol. Brain *9*, 5. https://doi.org/10.1186/s13041-016-0186-6.

Choi, S.H., Kim, Y.H., Hebisch, M., Sliwinski, C., Lee, S., D'Avanzo, C., Chen, H., Hooli, B., Asselin, C., Muffat, J., et al. (2014). A

three-dimensional human neural cell culture model of Alzheimer's disease. Nature *515*, 274–278. https://doi.org/10.1038/nature13800.

Chu, K., Kim, M., Jung, K.-H., Jeon, D., Lee, S.-T., Kim, J., Jeong, S.-W., Kim, S.U., Lee, S.K., Shin, H.-S., and Roh, J.K. (2004). Human neural stem cell transplantation reduces spontaneous recurrent seizures following pilocarpine-induced status epilepticus in adult rats. Brain Res. *1023*, 213–221. https://doi.org/10.1016/j.brainres.2004.07.045.

Chun, H., Im, H., Kang, Y.J., Kim, Y., Shin, J.H., Won, W., Lim, J., Ju, Y., Park, Y.M., Kim, S., et al. (2020). Severe reactive astrocytes precipitate pathological hallmarks of Alzheimer's disease via H2O2− production. Nat. Neurosci. *23*, 1555–1566. https://doi.org/10.1038/s41593-020-00735-y.

Colasante, G., Lignani, G., Rubio, A., Medrihan, L., Yekhlef, L., Sessa, A., Massimino, L., Giannelli, S.G., Sacchetti, S., Caiazzo, M., et al. (2015). Rapid Conversion of Fibroblasts into Functional Forebrain GABAergic Interneurons by Direct Genetic Reprogramming. Cell Stem Cell *17*, 719–734. https://doi.org/10.1016/j.stem.2015.09.002.

Contestabile, A., Fila, T., Ceccarelli, C., Bonasoni, P., Bonapace, L., Santini, D., Bartesaghi, R., and Ciani, E. (2007). Cell cycle alteration and decreased cell proliferation in the hippocampal dentate gyrus and in the neocortical germinal matrix of fetuses with Down syndrome and in Ts65Dn mice. Hippocampus *17*, 665–678. https://doi.org/10.1002/hipo.20308.

Cooper, A.A., Gitler, A.D., Cashikar, A., Haynes, C.M., Hill, K.J., Bhullar, B., Liu, K., Xu, K., Strathearn, K.E., Liu, F., et al. (2006). Alpha-synuclein blocks ER-Golgi traffic and Rab1 rescues neuron loss in Parkinson's models. Science *313*, 324–328. https://doi.org/10.1126/science.1129462.

Cunningham, M., Cho, J.-H., Leung, A., Savvidis, G., Ahn, S., Moon, M., Lee, P.K.J., Han, J.J., Azimi, N., Kim, K.-S., et al. (2014). hPSC-derived maturing GABAergic interneurons ameliorate seizures and abnormal behavior in epileptic mice. Cell Stem Cell *15*, 559–573. https://doi.org/10.1016/j.stem.2014.10.006.

de Curtis, M., Rossetti, A.O., Verde, D.V., van Vliet, E.A., and Ekdahl, C.T. (2021). Brain pathology in focal status epilepticus: evidence from experimental models. Neurosci. Biobehav. Rev. *131*, 834–846. https://doi.org/10.1016/j.neubiorev.2021.09.011.

Daher, J.P.L., Ying, M., Banerjee, R., McDonald, R.S., Hahn, M.D., Yang, L., Flint Beal, M., Thomas, B., Dawson, V.L., Dawson, T.M., and Moore, D.J. (2009). Conditional transgenic mice expressing C-terminally truncated human α-synuclein (αSyn119) exhibit reduced striatal dopamine without loss of nigrostriatal pathway dopaminergic neurons. Mol. Neurodegener. *4*, 34. https://doi.org/10.1186/1750-1326-4-34.

Dahle, A.J., and McCollister, F.P. (1986). Hearing and otologic disorders in children with Down syndrome. Am. J. Ment. Defic. *90*, 636–642.

Davis, M.F., Figueroa Velez, D.X., Guevarra, R.P., Yang, M.C., Habeeb, M., Carathedathu, M.C., and Gandhi, S.P. (2015). Inhibitory Neuron Transplantation into Adult Visual Cortex Creates a New Critical Period that Rescues Impaired Vision. Neuron *86*, 1055–1066. https://doi.org/10.1016/j.neuron.2015.03.062.

Davisson, M.T., Schmidt, C., and Akeson, E.C. (1990). Segmental trisomy of murine chromosome 16: a new model system for studying Down syndrome. Prog. Clin. Biol. Res. *360*, 263–280.

Dawson, T.M., Ko, H.S., and Dawson, V.L. (2010). Genetic animal models of Parkinson's disease. Neuron *66*, 646–661. https://doi.org/10.1016/j.neuron.2010.04.034.

Di Lullo, E., and Kriegstein, A.R. (2017). The use of brain organoids to investigate neural development and disease. Nat. Rev. Neurosci. *18*, 573–584. https://doi.org/10.1038/nrn.2017.107.

Dionísio, P.E.A., Oliveira, S.R., Amaral, J.S.J.D., and Rodrigues, C.M.P. (2019). Loss of Microglial Parkin Inhibits Necroptosis and Contributes to Neuroinflammation. Mol. Neurobiol. *56*, 2990–3004. https://doi.org/10.1007/s12035-018-1264-9.

Doi, D., Samata, B., Katsukawa, M., Kikuchi, T., Morizane, A., Ono, Y., Sekiguchi, K., Nakagawa, M., Parmar, M., and Takahashi, J. (2014). Isolation of human induced pluripotent stem cell-derived dopaminergic progenitors by cell sorting for successful transplantation. Stem Cell Rep. *2*, 337–350. https://doi.org/10.1016/j.stemcr.2014.01.013.

Donegan, J.J., Tyson, J.A., Branch, S.Y., Beckstead, M.J., Anderson, S.A., and Lodge, D.J. (2017). Stem cell-derived interneuron transplants as a treatment for schizophrenia: preclinical validation in a rodent model. Mol. Psychiatry *22*, 1492–1501. https://doi.org/10.1038/mp.2016.121.

Down, J.L. (1995). Observations on an ethnic classification of idiots. 1866. Ment. Retard. *33*, 54–56.

Dräger, N.M., Sattler, S.M., Huang, C.T.-L., Teter, O.M., Leng, K., Hashemi, S.H., Hong, J., Aviles, G., Clelland, C.D., Zhan, L., et al. (2022). A CRISPRi/a platform in human iPSC-derived microglia uncovers regulators of disease states. Nat. Neurosci. *25*, 1149–1162. https://doi.org/10.1038/s41593-022-01131-4.

Duffy, M.F., Collier, T.J., Patterson, J.R., Kemp, C.J., Luk, K.C., Tansey, M.G., Paumier, K.L., Kanaan, N.M., Fischer, D.L., Polinski, N.K., et al. (2018). Lewy body-like alpha-synuclein inclusions trigger reactive microgliosis prior to nigral degeneration. J. Neuroinflammation *15*, 129. https://doi.org/10.1186/s12974-018-1171-z.

Dunnett, S.B., Björklund, A., Stenevi, U., and Iversen, S.D. (1981). Behavioural recovery following transplantation of substantia nigra in rats subjected to 6-OHDA lesions of the nigrostriatal pathway. I. Unilateral lesions. Brain Res. *215*, 147–161. https://doi.org/10.1016/0006-8993(81)90498-4.

Dwyer, Z., Rudyk, C., Thompson, A., Farmer, K., Fenner, B., Fortin, T., Derksen, A., Sun, H., and Hayley, S.; CLINT Canadian LRRK2 in inflammation team (2020). Leucine-rich repeat kinase-2 (LRRK2) modulates microglial phenotype and dopaminergic neurodegeneration. Neurobiol. Aging *91*, 45–55. https://doi.org/10.1016/j.neurobiolaging.2020.02.017.

Ehringer, H., and Hornykiewicz, O. (1960). [Distribution of noradrenaline and dopamine (3-hydroxytyramine) in the human brain and their behavior in diseases of the extrapyramidal system]. Klin. Wochenschr. *38*, 1236–1239. https://doi.org/10.1007/BF01485901.

Eiraku, M., Watanabe, K., Matsuo-Takasaki, M., Kawada, M., Yonemura, S., Matsumura, M., Wataya, T., Nishiyama, A., Muguruma,

K., and Sasai, Y. (2008). Self-Organized Formation of Polarized Cortical Tissues from ESCs and Its Active Manipulation by Extrinsic Signals. Cell Stem Cell *3*, 519–532. https://doi.org/10.1016/j.stem.2008.09.002.

Espuny-Camacho, I., Michelsen, K.A., Gall, D., Linaro, D., Hasche, A., Bonnefont, J., Bali, C., Orduz, D., Bilheu, A., Herpoel, A., et al. (2013). Pyramidal neurons derived from human pluripotent stem cells integrate efficiently into mouse brain circuits in vivo. Neuron *77*, 440–456. https://doi.org/10.1016/j.neuron.2012.12.011.

Espuny-Camacho, I., Arranz, A.M., Fiers, M., Snellinx, A., Ando, K., Munck, S., Bonnefont, J., Lambot, L., Corthout, N., Omodho, L., et al. (2017). Hallmarks of Alzheimer's Disease in Stem-Cell-Derived Human Neurons Transplanted into Mouse Brain. Neuron *93*, 1066–1081.e8. https://doi.org/10.1016/j.neuron.2017.02.001.

Espuny-Camacho, I., Michelsen, K.A., Linaro, D., Bilheu, A., Acosta-Verdugo, S., Herpoel, A., Giugliano, M., Gaillard, A., and Vanderhaeghen, P. (2018). Human Pluripotent Stem-Cell-Derived Cortical Neurons Integrate Functionally into the Lesioned Adult Murine Visual Cortex in an Area-Specific Way. Cell Rep. *23*, 2732–2743. https://doi.org/10.1016/j.celrep.2018.04.094.

Fagiolini, M., and Hensch, T.K. (2000). Inhibitory threshold for critical-period activation in primary visual cortex. Nature *404*, 183–186. https://doi.org/10.1038/35004582.

Fahn, S., Oakes, D., Shoulson, I., Kieburtz, K., Rudolph, A., Lang, A., Olanow, C.W., Tanner, C., and Marek, K.; Parkinson Study Group (2004). Levodopa and the progression of Parkinson's disease. N. Engl. J. Med. *351*, 2498–2508. https://doi.org/10.1056/NEJMoa033447.

Falkner, S., Grade, S., Dimou, L., Conzelmann, K.-K., Bonhoeffer, T., Götz, M., and Hübener, M. (2016). Transplanted embryonic neurons integrate into adult neocortical circuits. Nature *539*, 248–253. https://doi.org/10.1038/nature20113.

Fan, L.Z., Kheifets, S., Böhm, U.L., Wu, H., Piatkevich, K.D., Xie, M.E., Parot, V., Ha, Y., Evans, K.E., Boyden, E.S., et al. (2020). All-Optical Electrophysiology Reveals the Role of Lateral Inhibition in Sensory Processing in Cortical Layer 1. Cell *180*, 521–535.e18. https://doi.org/10.1016/j.cell.2020.01.001.

Fattorelli, N., Martinez-Muriana, A., Wolfs, L., Geric, I., De Strooper, B., and Mancuso, R. (2021). Stem-cell-derived human microglia transplanted into mouse brain to study human disease. Nat. Protoc. *16*, 1013–1033. https://doi.org/10.1038/s41596-020-00447-4.

Fernandez, F., Morishita, W., Zuniga, E., Nguyen, J., Blank, M., Malenka, R.C., and Garner, C.C. (2007). Pharmacotherapy for cognitive impairment in a mouse model of Down syndrome. Nat. Neurosci. *10*, 411–413. https://doi.org/10.1038/nn1860.

Ferrer, I., and Gullotta, F. (1990). Down's syndrome and Alzheimer's disease: dendritic spine counts in the hippocampus. Acta Neuropathol. *79*, 680–685. https://doi.org/10.1007/BF00294247.

Freeman, S.B., Taft, L.F., Dooley, K.J., Allran, K., Sherman, S.L., Hassold, T.J., Khoury, M.J., and Saker, D.M. (1998). Population-based study of congenital heart defects in Down syndrome. Am. J. Med. Genet. *80*, 213–217.

Fulton, S.L., Wenderski, W., Lepack, A.E., Eagle, A.L., Fanutza, T., Bastle, R.M., Ramakrishnan, A., Hays, E.C., Neal, A., Bendl, J., et al. (2022). Rescue of deficits by Brwd1 copy number restoration in the Ts65Dn mouse model of Down syndrome. Nat. Commun. *13*, 6384. https://doi.org/10.1038/s41467-022-34200-0.

Furman, J.L., Sama, D.M., Gant, J.C., Beckett, T.L., Murphy, M.P., Bachstetter, A.D., Van Eldik, L.J., and Norris, C.M. (2012). Targeting Astrocytes Ameliorates Neurologic Changes in a Mouse Model of Alzheimer's Disease. J. Neurosci. *32*, 16129–16140. https://doi.org/10.1523/JNEUROSCI.2323-12.2012.

Gantner, C.W., de Luzy, I.R., Kauhausen, J.A., Moriarty, N., Niclis, J.C., Bye, C.R., Penna, V., Hunt, C.P.J., Ermine, C.M., Pouton, C.W., et al. (2020). Viral Delivery of GDNF Promotes Functional Integration of Human Stem Cell Grafts in Parkinson's Disease. Cell Stem Cell *26*, 511–526.e5. https://doi.org/10.1016/j.stem.2020.01.010.

Gaspard, N., Bouschet, T., Hourez, R., Dimidschstein, J., Naeije, G., van den Ameele, J., Espuny-Camacho, I., Herpoel, A., Passante, L., Schiffmann, S.N., et al. (2008). An intrinsic mechanism of corticogenesis from embryonic stem cells. Nature *455*, 351–357. https://doi.org/10.1038/nature07287.

Gelb, D.J., Oliver, E., and Gilman, S. (1999). Diagnostic criteria for Parkinson disease. Arch. Neurol. *56*, 33–39. https://doi.org/10.1001/archneur.56.1.33.

Geyer, M.A., Wilkinson, L.S., Humby, T., and Robbins, T.W. (1993). Isolation rearing of rats produces a deficit in prepulse inhibition of acoustic startle similar to that in schizophrenia. Biol. Psychiatry *34*, 361–372. https://doi.org/10.1016/0006-3223(93)90180-l.

Goddard, G.V., McIntyre, D.C., and Leech, C.K. (1969). A permanent change in brain function resulting from daily electrical stimulation. Exp. Neurol. *25*, 295–330. https://doi.org/10.1016/0014-4886(69)90128-9.

Goldberg, M.S., Pisani, A., Haburcak, M., Vortherms, T.A., Kitada, T., Costa, C., Tong, Y., Martella, G., Tscherter, A., Martins, A., et al. (2005). Nigrostriatal dopaminergic deficits and hypokinesia caused by inactivation of the familial Parkinsonism-linked gene DJ-1. Neuron *45*, 489–496. https://doi.org/10.1016/j.neuron.2005.01.041.

Gonzalez, C., Armijo, E., Bravo-Alegria, J., Becerra-Calixto, A., Mays, C.E., and 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.

Gorter, J.A., van Vliet, E.A., Aronica, E., and Lopes da Silva, F.H. (2001). Progression of spontaneous seizures after status epilepticus is associated with mossy fibre sprouting and extensive bilateral loss of hilar parvalbumin and somatostatin-immunoreactive neurons. Eur. J. Neurosci. *13*, 657–669. https://doi.org/10.1046/j.1460-9568.2001.01428.x.

Götz, J., Chen, F., van Dorpe, J., and Nitsch, R.M. (2001). Formation of neurofibrillary tangles in P301l tau transgenic mice induced by Abeta 42 fibrils. Science *293*, 1491–1495. https://doi.org/10.1126/science.1062097.

de Graaf, G., Buckley, F., and Skotko, B.G. (2017). Estimation of the number of people with Down syndrome in the United States. Genet. Med. *19*, 439–447. https://doi.org/10.1038/gim.2016.127.

Granholm, A.C., Sanders, L.A., and Crnic, L.S. (2000). Loss of Cholinergic Phenotype in Basal Forebrain Coincides with Cognitive Decline in a Mouse Model of Down's Syndrome. Exp. Neurol. *161*, 647–663. https://doi.org/10.1006/exnr.1999.7289.

Grealish, S., Diguet, E., Kirkeby, A., Mattsson, B., Heuer, A., Bramoulle, Y., Van Camp, N., Perrier, A.L., Hantraye, P., Björklund, A., and Parmar, M. (2014). Human ESC-derived dopamine neurons show similar preclinical efficacy and potency to fetal neurons when grafted in a rat model of Parkinson's disease. Cell Stem Cell *15*, 653–665. https://doi.org/10.1016/j.stem.2014.09.017.

Green, M.C., and Sidman, R.L. (1962). TOTTERING—A NEUROMUSCULAR MUTATION IN THE MOUSE: And Its Linkage With Oligosyndactylism. J. Hered. *53*, 233–237. https://doi.org/10.1093/oxfordjournals.jhered.a107180.

Griffin, W.S., Sheng, J.G., McKenzie, J.E., Royston, M.C., Gentleman, S.M., Brumback, R.A., Cork, L.C., Del Bigio, M.R., Roberts, G.W., and Mrak, R.E. (1998). Life-long overexpression of S100beta in Down's syndrome: implications for Alzheimer pathogenesis. Neurobiol. Aging *19*, 401–405. https://doi.org/10.1016/s0197-4580(98)00074-8.

Grossman, N., Bono, D., Dedic, N., Kodandaramaiah, S.B., Rudenko, A., Suk, H.-J., Cassara, A.M., Neufeld, E., Kuster, N., Tsai, L.-H., et al. (2017). Noninvasive deep brain stimulation via temporally interfering electric fields. Cell *169*, 1029–1041.e16. https://doi.org/10.1016/j.cell.2017.05.024.

Gu, X.-L., Long, C.-X., Sun, L., Xie, C., Lin, X., and Cai, H. (2010). Astrocytic expression of Parkinson's disease-related A53T alpha-synuclein causes neurodegeneration in mice. Mol. Brain *3*, 12. https://doi.org/10.1186/1756-6606-3-12.

Guedj, F., Kane, E., Bishop, L.A., Pennings, J.L.A., Herault, Y., and Bianchi, D.W. (2023). The Impact of Mmu17 Non-Hsa21 Orthologous Genes in the Ts65Dn Mouse Model of Down Syndrome: The Gold Standard Refuted. Biol. Psychiatry *94*, 84–97. https://doi.org/10.1016/j.biopsych.2023.02.012.

Guidi, S., Bonasoni, P., Ceccarelli, C., Santini, D., Gualtieri, F., Ciani, E., and Bartesaghi, R. (2008). Neurogenesis impairment and increased cell death reduce total neuron number in the hippocampal region of fetuses with Down syndrome. Brain Pathol. *18*, 180–197. https://doi.org/10.1111/j.1750-3639.2007.00113.x.

Habib, N., McCabe, C., Medina, S., Varshavsky, M., Kitsberg, D., Dvir-Szternfeld, R., Green, G., Dionne, D., Nguyen, L., Marshall, J.L., et al. (2020). Disease-associated astrocytes in Alzheimer's disease and aging. Nat. Neurosci. *23*, 701–706. https://doi.org/10.1038/s41593-020-0624-8.

Hanchate, N.K., Lee, E.J., Ellis, A., Kondoh, K., Kuang, D., Basom, R., Trapnell, C., and Buck, L.B. (2020). Connect-seq to superimpose molecular on anatomical neural circuit maps. Proc. Natl. Acad. Sci. USA *117*, 4375–4384. https://doi.org/10.1073/pnas.1912176117.

Hasselmann, J., Coburn, M.A., England, W., Figueroa Velez, D.X., Kiani Shabestari, S., Tu, C.H., McQuade, A., Kolahdouzan, M., Echeverria, K., Claes, C., et al. (2019). Development of a Chimeric Model to Study and Manipulate Human Microglia In Vivo. Neuron *103*, 1016–1033.e10. https://doi.org/10.1016/j.neuron.2019.07.002.

Hattori, M., Fujiyama, A., Taylor, T.D., Watanabe, H., Yada, T., Park, H.S., Toyoda, A., Ishii, K., Totoki, Y., Choi, D.K., et al. (2000). The DNA sequence of human chromosome 21. Nature *405*, 311–319. https://doi.org/10.1038/35012518.

Hauser, W.A., and Kurland, L.T. (1975). The epidemiology of epilepsy in Rochester, Minnesota, 1935 through 1967. Epilepsia *16*, 1–66. https://doi.org/10.1111/j.1528-1157.1975.tb04721.x.

Hauser, W.A., Annegers, J.F., and Kurland, L.T. (1993). Incidence of epilepsy and unprovoked seizures in Rochester, Minnesota: 1935-1984. Epilepsia *34*, 453–468. https://doi.org/10.1111/j.1528-1157.1993.tb02586.x.

Higurashi, N., Uchida, T., Lossin, C., Misumi, Y., Okada, Y., Akamatsu, W., Imaizumi, Y., Zhang, B., Nabeshima, K., Mori, M.X., et al. (2013). A human Dravet syndrome model from patient induced pluripotent stem cells. Mol. Brain *6*, 19. https://doi.org/10.1186/1756-6606-6-19.

Hochbaum, D.R., Zhao, Y., Farhi, S.L., Klapoetke, N., Werley, C.A., Kapoor, V., Zou, P., Kralj, J.M., Maclaurin, D., Smedemark-Margulies, N., et al. (2014). All-optical electrophysiology in mammalian neurons using engineered microbial rhodopsins. Nat. Methods *11*, 825–833. https://doi.org/10.1038/nmeth.3000.

Holtzman, D.M., Santucci, D., Kilbridge, J., Chua-Couzens, J., Fontana, D.J., Daniels, S.E., Johnson, R.M., Chen, K., Sun, Y., Carlson, E., et al. (1996). Developmental abnormalities and age-related neurodegeneration in a mouse model of Down syndrome. Proc. Natl. Acad. Sci. USA *93*, 13333–13338. https://doi.org/10.1073/pnas.93.23.13333.

Hu, N.-W., Corbett, G.T., Moore, S., Klyubin, I., O'Malley, T.T., Walsh, D.M., Livesey, F.J., and Rowan, M.J. (2018). Extracellular Forms of Aβ and Tau from iPSC Models of Alzheimer's Disease Disrupt Synaptic Plasticity. Cell Rep. *23*, 1932–1938. https://doi.org/10.1016/j.celrep.2018.04.040.

Hulshoff Pol, H.E., Hoek, H.W., Susser, E., Brown, A.S., Dingemans, A., Schnack, H.G., van Haren, N.E., Pereira Ramos, L.M., Gispen-de Wied, C.C., and Kahn, R.S. (2000). Prenatal exposure to famine and brain morphology in schizophrenia. Am. J. Psychiatry *157*, 1170–1172. https://doi.org/10.1176/appi.ajp.157.7.1170.

Hunter, C.L., Isacson, O., Nelson, M., Bimonte-Nelson, H., Seo, H., Lin, L., Ford, K., Kindy, M.S., and Granholm, A.-C. (2003). Regional alterations in amyloid precursor protein and nerve growth factor across age in a mouse model of Down's syndrome. Neurosci. Res. *45*, 437–445. https://doi.org/10.1016/S0168-0102(03)00005-1.

Huo, H.-Q., Qu, Z.-Y., Yuan, F., Ma, L., Yao, L., Xu, M., Hu, Y., Ji, J., Bhattacharyya, A., Zhang, S.-C., and Liu, Y. (2018). Modeling Down Syndrome with Patient iPSCs Reveals Cellular and Migration Deficits of GABAergic Neurons. Stem Cell Rep. *10*, 1251–1266. https://doi.org/10.1016/j.stemcr.2018.02.001.

Hurtado, D.E., Molina-Porcel, L., Iba, M., Aboagye, A.K., Paul, S.M., Trojanowski, J.Q., and Lee, V.M.-Y. (2010). A{beta} accelerates the spatiotemporal progression of tau pathology and augments tau amyloidosis in an Alzheimer mouse model. Am. J. Pathol. *177*, 1977–1988. https://doi.org/10.2353/ajpath.2010.100346.

Itagaki, S., McGeer, P.L., Akiyama, H., Zhu, S., and Selkoe, D. (1989). Relationship of microglia and astrocytes to amyloid deposits of Alzheimer disease. J. Neuroimmunol. *24*, 173–182. https://doi.org/10.1016/0165-5728(89)90115-x.

Jefferys, J.G. (1994). Experimental neurobiology of epilepsies. Curr. Opin. Neurol. *7*, 113–122. https://doi.org/10.1097/00019052-199404000-00007.

Jiang, P., Chen, C., Wang, R., Chechneva, O.V., Chung, S.-H., Rao, M.S., Pleasure, D.E., Liu, Y., Zhang, Q., and Deng, W. (2013). hESC-derived Olig2+ progenitors generate a subtype of astroglia with protective effects against ischaemic brain injury. Nat. Commun. *4*, 2196. https://doi.org/10.1038/ncomms3196.

Jiao, J., Yang, Y., Shi, Y., Chen, J., Gao, R., Fan, Y., Yao, H., Liao, W., Sun, X.-F., and Gao, S. (2013). Modeling Dravet syndrome using induced pluripotent stem cells (iPSCs) and directly converted neurons. Hum. Mol. Genet. *22*, 4241–4252. https://doi.org/10.1093/hmg/ddt275.

Jin, M., Xu, R., Wang, L., Alam, M.M., Ma, Z., Zhu, S., Martini, A.C., Jadali, A., Bernabucci, M., Xie, P., et al. (2022). Type-I-interferon signaling drives microglial dysfunction and senescence in human iPSC models of Down syndrome and Alzheimer's disease. Cell Stem Cell *29*, 1135–1153.e8. https://doi.org/10.1016/j.stem.2022.06.007.

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., et al. (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.

Jones, D.L., and Baraban, S.C. (2007). Characterization of Inhibitory Circuits in the Malformed Hippocampus of *Lis1* Mutant Mice. J. Neurophysiol. *98*, 2737–2746. https://doi.org/10.1152/jn.00938.2007.

Jucker, M. (2010). The benefits and limitations of animal models for translational research in neurodegenerative diseases. Nat. Med. *16*, 1210–1214. https://doi.org/10.1038/nm.2224.

Kathuria, A., Lopez-Lengowski, K., Jagtap, S.S., McPhie, D., Perlis, R.H., Cohen, B.M., and Karmacharya, R. (2020). Transcriptomic Landscape and Functional Characterization of Induced Pluripotent Stem Cell-Derived Cerebral Organoids in Schizophrenia. JAMA Psychiatr. *77*, 745–754. https://doi.org/10.1001/jamapsychiatry.2020.0196.

Kawatani, K., Nambara, T., Nawa, N., Yoshimatsu, H., Kusakabe, H., Hirata, K., Tanave, A., Sumiyama, K., Banno, K., Taniguchi, H., et al. (2021). A human isogenic iPSC-derived cell line panel identifies major regulators of aberrant astrocyte proliferation in Down syndrome. Commun. Biol. *4*, 730–745. https://doi.org/10.1038/s42003-021-02242-7.

Kelley, K.W., and Pașca, S.P. (2022). Human brain organogenesis: Toward a cellular understanding of development and disease. Cell *185*, 42–61. https://doi.org/10.1016/j.cell.2021.10.003.

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., et al. (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.

Kesslak, J.P., Nagata, S.F., Lott, I., and Nalcioglu, O. (1994). Magnetic resonance imaging analysis of age-related changes in the brains of individuals with Down's syndrome. Neurology *44*, 1039–1045. https://doi.org/10.1212/wnl.44.6.1039.

Khachaturian, Z.S. (1985). Diagnosis of Alzheimer's disease. Arch. Neurol. *42*, 1097–1105. https://doi.org/10.1001/archneur.1985.04060100083029.

Kikuchi, T., Morizane, A., Doi, D., Magotani, H., Onoe, H., Hayashi, T., Mizuma, H., Takara, S., Takahashi, R., Inoue, H., et al. (2017). Human iPS cell-derived dopaminergic neurons function in a primate Parkinson's disease model. Nature *548*, 592–596. https://doi.org/10.1038/nature23664.

Kim, H., Park, H.J., Choi, H., Chang, Y., Park, H., Shin, J., Kim, J., Lengner, C.J., Lee, Y.K., and 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, H.W., Quan, Z., Kim, Y.-B., Cheong, E., Kim, H.D., Cho, M., Jang, J., Yoo, Y.R., Lee, J.S., Kim, J.H., et al. (2018). Differential effects on sodium current impairments by distinct SCN1A mutations in GABAergic neurons derived from Dravet syndrome patients. Brain Dev. *40*, 287–298. https://doi.org/10.1016/j.braindev.2017.12.002.

Kim, J.H., Guimaraes, P.O., Shen, M.Y., Masukawa, L.M., and Spencer, D.D. (1990). Hippocampal neuronal density in temporal lobe epilepsy with and without gliomas. Acta Neuropathol. *80*, 41–45. https://doi.org/10.1007/BF00294220.

Kim, T.W., Piao, J., Koo, S.Y., Kriks, S., Chung, S.Y., Betel, D., Socci, N.D., Choi, S.J., Zabierowski, S., Dubose, B.N., et al. (2021). Biphasic Activation of WNT Signaling Facilitates the Derivation of Midbrain Dopamine Neurons from hESCs for Translational Use. Cell Stem Cell *28*, 343–355.e5. https://doi.org/10.1016/j.stem.2021.01.005.

Kirkeby, A., Grealish, S., Wolf, D.A., Nelander, J., Wood, J., Lundblad, M., Lindvall, O., and 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. https://doi.org/10.1016/j.celrep.2012.04.009.

Kiskinis, E., Kralj, J.M., Zou, P., Weinstein, E.N., Zhang, H., Tsioras, K., Wiskow, O., Ortega, J.A., Eggan, K., and Cohen, A.E. (2018). All-Optical Electrophysiology for High-Throughput Functional Characterization of a Human iPSC-Derived Motor Neuron Model of ALS. Stem Cell Rep. *10*, 1991–2004. https://doi.org/10.1016/j.stemcr.2018.04.020.

Kitada, T., Tong, Y., Gautier, C.A., and Shen, J. (2009). Absence of nigral degeneration in aged parkin/DJ-1/PINK1 triple knockout mice. J. Neurochem. *111*, 696–702. https://doi.org/10.1111/j.1471-4159.2009.06350.x.

Kondo, T., Asai, M., Tsukita, K., Kutoku, Y., Ohsawa, Y., Sunada, Y., Imamura, K., Egawa, N., Yahata, N., Okita, K., et al. (2013). Modeling Alzheimer's disease with iPSCs reveals stress phenotypes associated with intracellular Aβ and differential drug responsiveness. Cell Stem Cell *12*, 487–496. https://doi.org/10.1016/j.stem.2013.01.009.

Krencik, R., Weick, J.P., Liu, Y., Zhang, Z.-J., and Zhang, S.-C. (2011). Specification of transplantable astroglial subtypes from human pluripotent stem cells. Nat. Biotechnol. *29*, 528–534. https://doi.org/10.1038/nbt.1877.

Kriks, S., Shim, J.-W., Piao, J., Ganat, Y.M., Wakeman, D.R., Xie, Z., Carrillo-Reid, L., Auyeung, G., Antonacci, C., Buch, A., et al. (2011). Dopamine neurons derived from human ES cells efficiently engraft in animal models of Parkinson's disease. Nature *480*, 547–551. https://doi.org/10.1038/nature10648.

LaFerla, F.M., and Green, K.N. (2012). Animal Models of Alzheimer Disease. Cold Spring Harb. Perspect. Med. *2*, a006320. https://doi.org/10.1101/cshperspect.a006320.

Lambert, M.P., Barlow, A.K., Chromy, B.A., Edwards, C., Freed, R., Liosatos, M., Morgan, T.E., Rozovsky, I., Trommer, B., Viola, K.L., et al. (1998). Diffusible, nonfibrillar ligands derived from Abeta1-42 are potent central nervous system neurotoxins. Proc. Natl. Acad. Sci. USA *95*, 6448–6453. https://doi.org/10.1073/pnas.95.11.6448.

Lancaster, M.A., Renner, M., Martin, C.-A., Wenzel, D., Bicknell, L.S., Hurles, M.E., Homfray, T., Penninger, J.M., Jackson, A.P., and Knoblich, J.A. (2013). Cerebral organoids model human brain development and microcephaly. Nature *501*, 373–379. https://doi.org/10.1038/nature12517.

de Lanerolle, N.C., Kim, J.H., Robbins, R.J., and Spencer, D.D. (1989). Hippocampal interneuron loss and plasticity in human temporal lobe epilepsy. Brain Res. *495*, 387–395. https://doi.org/10.1016/0006-8993(89)90234-5.

Laperle, A.H., Sances, S., Yucer, N., Dardov, V.J., Garcia, V.J., Ho, R., Fulton, A.N., Jones, M.R., Roxas, K.M., Avalos, P., et al. (2020). iPSC modeling of young-onset Parkinson's disease reveals a molecular signature of disease and novel therapeutic candidates. Nat. Med. *26*, 289–299. https://doi.org/10.1038/s41591-019-0739-1.

Larsen, K.B., Laursen, H., Graem, N., Samuelsen, G.B., Bogdanovic, N., and Pakkenberg, B. (2008). Reduced cell number in the neocortical part of the human fetal brain in Down syndrome. Ann. Anat. *190*, 421–427. https://doi.org/10.1016/j.aanat.2008.05.007.

Lee, H., Yun, S., Kim, I.-S., Lee, I.-S., Shin, J.E., Park, S.C., Kim, W.-J., and Park, K.I. (2014). Human fetal brain-derived neural stem/progenitor cells grafted into the adult epileptic brain restrain seizures in rat models of temporal lobe epilepsy. PLoS One *9*, e104092. https://doi.org/10.1371/journal.pone.0104092.

Lee, J.H., Han, J.-H., Kim, H., Park, S.M., Joe, E.-H., and Jou, I. (2019). Parkinson's disease-associated LRRK2-G2019S mutant acts through regulation of SERCA activity to control ER stress in astrocytes. Acta Neuropathol. Commun. *7*, 68. https://doi.org/10.1186/s40478-019-0716-4.

Lee, S.E., Duran-Martinez, M., Khantsis, S., Bianchi, D.W., and Guedj, F. (2020). Challenges and Opportunities for Translation of Therapies to Improve Cognition in Down Syndrome. Trends Mol. Med. *26*, 150–169. https://doi.org/10.1016/j.molmed.2019.10.001.

Lee, Y., Stevens, D.A., Kang, S.-U., Jiang, H., Lee, Y.-I., Ko, H.S., Scarffe, L.A., Umanah, G.E., Kang, H., Ham, S., et al. (2017). PINK1 Primes Parkin-Mediated Ubiquitination of PARIS in Dopaminergic Neuronal Survival. Cell Rep. *18*, 918–932. https://doi.org/10.1016/j.celrep.2016.12.090.

Lejeune, J., Gautier, M., and Turpin, R. (1959). [Study of somatic chromosomes from 9 mongoloid children]. C. R. Hebd. Seances Acad. Sci. *248*, 1721–1722.

Levin, B.E., Llabre, M.M., and Weiner, W.J. (1989). Cognitive impairments associated with early Parkinson's disease. Neurology *39*, 557–561. https://doi.org/10.1212/wnl.39.4.557.

Lewis, J., Dickson, D.W., Lin, W.L., Chisholm, L., Corral, A., Jones, G., Yen, S.H., Sahara, N., Skipper, L., Yager, D., et al. (2001). Enhanced neurofibrillary degeneration in transgenic mice expressing mutant tau and APP. Science *293*, 1487–1491. https://doi.org/10.1126/science.1058189.

Li, J., Pan, L., Pembroke, W.G., Rexach, J.E., Godoy, M.I., Condro, M.C., Alvarado, A.G., Harteni, M., Chen, Y.-W., Stiles, L., et al. (2021). Conservation and divergence of vulnerability and responses to stressors between human and mouse astrocytes. Nat. Commun. *12*, 3958. https://doi.org/10.1038/s41467-021-24232-3.

Li, Y., Liu, W., Oo, T.F., Wang, L., Tang, Y., Jackson-Lewis, V., Zhou, C., Geghman, K., Bogdanov, M., Przedborski, S., et al. (2009). Mutant LRRK2(R1441G) BAC transgenic mice recapitulate cardinal features of Parkinson's disease. Nat. Neurosci. *12*, 826–828. https://doi.org/10.1038/nn.2349.

Li, Z., Yu, T., Morishima, M., Pao, A., LaDuca, J., Conroy, J., Nowak, N., Matsui, S.-I., Shiraishi, I., and Yu, Y.E. (2007). Duplication of the entire 22.9 Mb human chromosome 21 syntenic region on mouse chromosome 16 causes cardiovascular and gastrointestinal abnormalities. Hum. Mol. Genet. *16*, 1359–1366. https://doi.org/10.1093/hmg/ddm086.

Liang, W., Hou, Y., Huang, W., Wang, Y., Jiang, T., Huang, X., Wang, Z., Wu, F., Zheng, J., Zhang, J., et al. (2022). Loss of schizophrenia-related miR-501-3p in mice impairs sociability and memory by enhancing mGluR5-mediated glutamatergic transmission. Sci. Adv. *8*, eabn7357. https://doi.org/10.1126/sciadv.abn7357.

Liao, M.-C., Muratore, C.R., Gierahn, T.M., Sullivan, S.E., Srikanth, P., De Jager, P.L., Love, J.C., and Young-Pearse, T.L. (2016). Single-Cell Detection of Secreted Aβ and sAPPα from Human IPSC-Derived Neurons and Astrocytes. J. Neurosci. *36*, 1730–1746. https://doi.org/10.1523/JNEUROSCI.2735-15.2016.

Liddelow, S.A., Guttenplan, K.A., Clarke, L.E., Bennett, F.C., Bohlen, C.J., Schirmer, L., Bennett, M.L., Münch, A.E., Chung, W.-S., Peterson, T.C., et al. (2017). Neurotoxic reactive astrocytes are induced by activated microglia. Nature *541*, 481–487. https://doi.org/10.1038/nature21029.

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., et al. (2018). APOE4 Causes Widespread Molecular and Cellular Alterations Associated with Alzheimer's Disease Phenotypes in Human iPSC-Derived Brain Cell Types. Neuron *98*, 1141–1154.e7. https://doi.org/10.1016/j.neuron.2018.05.008.

Linaro, D., Vermaercke, B., Iwata, R., Ramaswamy, A., Libé-Philippot, B., Boubakar, L., Davis, B.A., Wierda, K., Davie, K., Poovathingal, S., et al. (2019). Xenotransplanted Human Cortical Neurons Reveal Species-Specific Development and Functional Integration into Mouse Visual Circuits. Neuron *104*, 972–986.e6. https://doi.org/10.1016/j.neuron.2019.10.002.

Liu, J., Gao, C., Chen, W., Ma, W., Li, X., Shi, Y., Zhang, H., Zhang, L., Long, Y., Xu, H., et al. (2016). CRISPR/Cas9 facilitates investigation of neural circuit disease using human iPSCs: mechanism of epilepsy caused by an SCN1A loss-of-function mutation. Transl. Psychiatry *6*, e703. https://doi.org/10.1038/tp.2015.203.

Liu, Y., Lopez-Santiago, L.F., Yuan, Y., Jones, J.M., Zhang, H., O'Malley, H.A., Patino, G.A., O'Brien, J.E., Rusconi, R., Gupta, A., et al. (2013). Dravet syndrome patient-derived neurons suggest a novel epilepsy mechanism. Ann. Neurol. *74*, 128–139. https://doi.org/10.1002/ana.23897.

Liu, Y., Borel, C., Li, L., Müller, T., Williams, E.G., Germain, P.-L., Buljan, M., Sajic, T., Boersema, P.J., Shao, W., et al. (2017). Systematic proteome and proteostasis profiling in human Trisomy 21 fibroblast cells. Nat. Commun. *8*, 1212. https://doi.org/10.1038/s41467-017-01422-6.

Lodge, D.J., and Grace, A.A. (2007). Aberrant hippocampal activity underlies the dopamine dysregulation in an animal model of schizophrenia. J. Neurosci. *27*, 11424–11430. https://doi.org/10.1523/JNEUROSCI.2847-07.2007.

López-Tobón, A., Shyti, R., Villa, C.E., Cheroni, C., Fuentes-Bravo, P., Trattaro, S., Caporale, N., Troglio, F., Tenderini, E., Mihailovich, M., et al. (2023). GTF2I dosage regulates neuronal differentiation and social behavior in 7q11.23 neurodevelopmental disorders. Sci. Adv. *9*, eadh2726. https://doi.org/10.1126/sciadv.adh2726.

Lozano, A.M. (2017). Waving Hello to Noninvasive Deep-Brain Stimulation. N. Engl. J. Med. *377*, 1096–1098. https://doi.org/10.1056/NEJMcibr1707165.

Lu, H.-E., Yang, Y.-C., Chen, S.-M., Su, H.-L., Huang, P.-C., Tsai, M.-S., Wang, T.-H., Tseng, C.-P., and Hwang, S.-M. (2013). Modeling neurogenesis impairment in down syndrome with induced pluripotent stem cells from Trisomy 21 amniotic fluid cells. Exp. Cell Res. *319*, 498–505. https://doi.org/10.1016/j.yexcr.2012.09.017.

Ma, T.M., Abazyan, S., Abazyan, B., Nomura, J., Yang, C., Seshadri, S., Sawa, A., Snyder, S.H., and Pletnikov, M.V. (2013). Pathogenic disruption of DISC1-serine racemase binding elicits schizophrenia-like behavior via D-serine depletion. Mol. Psychiatry *18*, 557–567. https://doi.org/10.1038/mp.2012.97.

MacMahon Copas, A.N., McComish, S.F., Fletcher, J.M., and Caldwell, M.A. (2021). The Pathogenesis of Parkinson's Disease: A Complex Interplay Between Astrocytes, Microglia, and T Lymphocytes? Front. Neurol. *12*, 666737. https://doi.org/10.3389/fneur.2021.666737.

Mahajani, S., Raina, A., Fokken, C., Kügler, S., and Bähr, M. (2019). Homogenous generation of dopaminergic neurons from multiple hiPSC lines by transient expression of transcription factors. Cell Death Dis. *10*, 898–913. https://doi.org/10.1038/s41419-019-2133-9.

Mancuso, R., Van Den Daele, J., Fattorelli, N., Wolfs, L., Balusu, S., Burton, O., Liston, A., Sierksma, A., Fourne, Y., Poovathingal, S., et al. (2019). Stem-cell-derived human microglia transplanted in mouse brain to study human disease. Nat. Neurosci. *22*, 2111–2116. https://doi.org/10.1038/s41593-019-0525-x.

Mancuso, R., Fattorelli, N., Martinez-Muriana, A., Davis, E., Wolfs, L., Daele, J.V.D., Geric, I., Preman, P., Serneels, L., Poovathingal, S., et al. (2022). A Multi-Pronged Human Microglia Response to Alzheimer's Disease Aβ Pathology. Preprint at bioRxiv. https://doi.org/10.1101/2022.07.07.499139.

Mansour, A.A., Gonçalves, J.T., Bloyd, C.W., Li, H., Fernandes, S., Quang, D., Johnston, S., Parylak, S.L., Jin, X., and 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.

Marchetto, M.C.N., Carromeu, C., Acab, A., Yu, D., Yeo, G.W., Mu, Y., Chen, G., Gage, F.H., and Muotri, A.R. (2010). A Model for Neural Development and Treatment of Rett Syndrome Using Human Induced Pluripotent Stem Cells. Cell *143*, 527–539. https://doi.org/10.1016/j.cell.2010.10.016.

Margerison, J.H., and Corsellis, J.A. (1966). Epilepsy and the temporal lobes. A clinical, electroencephalographic and neuropathological study of the brain in epilepsy, with particular reference to the temporal lobes. Brain *89*, 499–530. https://doi.org/10.1093/brain/89.3.499.

Marin-Padilla, M. (1976). Pyramidal cell abnormalities in the motor cortex of a child with Down's syndrome. A Golgi study. J. Comp. Neurol. *167*, 63–81. https://doi.org/10.1002/cne.901670105.

Mätlik, K., Garton, D.R., Montaño-Rodríguez, A.R., Olfat, S., Eren, F., Casserly, L., Damdimopoulos, A., Panhelainen, A., Porokuokka, L.L., Kopra, J.J., et al. (2022). Elevated endogenous GDNF induces altered dopamine signalling in mice and correlates with clinical severity in schizophrenia. Mol. Psychiatry *27*, 3247–3261. https://doi.org/10.1038/s41380-022-01554-2.

Matsumoto, H., and Marsan, C.A. (1964). CORTICAL CELLULAR PHENOMENA IN EXPERIMENTAL EPILEPSY: ICTAL MANIFESTATIONS. Exp. Neurol. *9*, 305–326. https://doi.org/10.1016/0014-4886(64)90026-3.

McCarron, M., McCallion, P., Reilly, E., and Mulryan, N. (2014). A prospective 14-year longitudinal follow-up of dementia in persons with Down syndrome. J. Intellect. Disabil. Res. *58*, 61–70. https://doi.org/10.1111/jir.12074.

McCormick, D.A., and Contreras, D. (2001). On the cellular and network bases of epileptic seizures. Annu. Rev. Physiol. *63*, 815–846. https://doi.org/10.1146/annurev.physiol.63.1.815.

McCullumsmith, R.E., Hammond, J.H., Shan, D., and Meador-Woodruff, J.H. (2014). Postmortem Brain: An Underutilized Substrate for Studying Severe Mental Illness. Neuropsychopharmacology *39*, 65–87. https://doi.org/10.1038/npp.2013.239.

Meharena, H.S., Marco, A., Dileep, V., Lockshin, E.R., Akatsu, G.Y., Mullahoo, J., Watson, L.A., Ko, T., Guerin, L.N., Abdurrob, F., et al. (2022). Down-syndrome-induced senescence disrupts the nuclear architecture of neural progenitors. Cell Stem Cell *29*, 116–130.e7. https://doi.org/10.1016/j.stem.2021.12.002.

Michelsen, K.A., Acosta-Verdugo, S., Benoit-Marand, M., Espuny-Camacho, I., Gaspard, N., Saha, B., Gaillard, A., and Vanderhaeghen, P. (2015). Area-specific reestablishment of damaged circuits in the adult cerebral cortex by cortical neurons derived from mouse embryonic stem cells. Neuron *85*, 982–997. https://doi.org/10.1016/j.neuron.2015.02.001.

Mito, T., and Becker, L.E. (1993). Developmental changes of S-100 protein and glial fibrillary acidic protein in the brain in Down syndrome. Exp. Neurol. *120*, 170–176. https://doi.org/10.1006/exnr.1993.1052.

Moghaddam, B., Adams, B., Verma, A., and Daly, D. (1997). Activation of glutamatergic neurotransmission by ketamine: a novel step in the pathway from NMDA receptor blockade to dopaminergic

and cognitive disruptions associated with the prefrontal cortex. J. Neurosci. *17*, 2921–2927. https://doi.org/10.1523/JNEUROSCI.17-08-02921.1997.

Monzel, A.S., Smits, L.M., Hemmer, K., Hachi, S., Moreno, E.L., van Wuellen, T., Jarazo, J., Walter, J., Brüggemann, I., Boussaad, I., et al. (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.

Muratore, C.R., Rice, H.C., Srikanth, P., Callahan, D.G., Shin, T., Benjamin, L.N.P., Walsh, D.M., Selkoe, D.J., and Young-Pearse, T.L. (2014). The familial Alzheimer's disease APPV717I mutation alters APP processing and Tau expression in iPSC-derived neurons. Hum. Mol. Genet. *23*, 3523–3536. https://doi.org/10.1093/hmg/ddu064.

Murray, R.M., and Lewis, S.W. (1988). Is schizophrenia a neurodevelopmental disorder? Br. Med. J. *296*, 63. https://doi.org/10.1136/bmj.296.6614.63.

Nash, Y., Schmukler, E., Trudler, D., Pinkas-Kramarski, R., and Frenkel, D. (2017). DJ-1 deficiency impairs autophagy and reduces alpha-synuclein phagocytosis by microglia. J. Neurochem. *143*, 584–594. https://doi.org/10.1111/jnc.14222.

Nausieda, P.A., Weiner, W.J., Kaplan, L.R., Weber, S., and Klawans, H.L. (1982). Sleep disruption in the course of chronic levodopa therapy: an early feature of the levodopa psychosis. Clin. Neuropharmacol. *5*, 183–194. https://doi.org/10.1097/00002826-198205020-00003.

Negraes, P.D., Trujillo, C.A., Yu, N.-K., Wu, W., Yao, H., Liang, N., Lautz, J.D., Kwok, E., McClatchy, D., Diedrich, J., et al. (2021). Altered network and rescue of human neurons derived from individuals with early-onset genetic epilepsy. Mol. Psychiatry *26*, 7047–7068. https://doi.org/10.1038/s41380-021-01104-2.

Nestler, E.J., and Hyman, S.E. (2010). Animal models of neuropsychiatric disorders. Nat. Neurosci. *13*, 1161–1169. https://doi.org/10.1038/nn.2647.

Neuhof, H. (1923). The Transplantation of Tissues (New York: D. Appleton and Company).

Ni, P., Noh, H., Park, G.-H., Shao, Z., Guan, Y., Park, J.M., Yu, S., Park, J.S., Coyle, J.T., Weinberger, D.R., et al. (2020). iPSC-derived homogeneous populations of developing schizophrenia cortical interneurons have compromised mitochondrial function. Mol. Psychiatry *25*, 2873–2888. https://doi.org/10.1038/s41380-019-0423-3.

Noè, F., Cattalini, A., Vila Verde, D., Alessi, C., Colciaghi, F., Figini, M., Zucca, I., and de Curtis, M. (2019). Epileptiform activity contralateral to unilateral hippocampal sclerosis does not cause the expression of brain damage markers. Epilepsia *60*, 1184–1199. https://doi.org/10.1111/epi.15611.

Oksanen, M., Petersen, A.J., Naumenko, N., Puttonen, K., Lehtonen, Š., Gubert Olivé, M., Shakirzyanova, A., Leskelä, S., Sarajärvi, T., Viitanen, M., et al. (2017). PSEN1 Mutant iPSC-Derived Model Reveals Severe Astrocyte Pathology in Alzheimer's Disease. Stem Cell Rep. *9*, 1885–1897. https://doi.org/10.1016/j.stemcr.2017.10.016.

Olmos-Serrano, J.L., Tyler, W.A., Cabral, H.J., and Haydar, T.F. (2016). Longitudinal measures of cognition in the Ts65Dn mouse: Refining windows and defining modalities for therapeutic intervention in Down syndrome. Exp. Neurol. *279*, 40–56. https://doi.org/10.1016/j.expneurol.2016.02.005.

Ovchinnikov, D.A., Korn, O., Virshup, I., Wells, C.A., and Wolvetang, E.J. (2018). The Impact of APP on Alzheimer-like Pathogenesis and Gene Expression in Down Syndrome iPSC-Derived Neurons. Stem Cell Rep. *11*, 32–42. https://doi.org/10.1016/j.stemcr.2018.05.004.

Panagiotakopoulou, V., Ivanyuk, D., De Cicco, S., Haq, W., Arsić, A., Yu, C., Messelodi, D., Oldrati, M., Schöndorf, D.C., Perez, M.-J., et al. (2020). Interferon-γ signaling synergizes with LRRK2 in neurons and microglia derived from human induced pluripotent stem cells. Nat. Commun. *11*, 5163. https://doi.org/10.1038/s41467-020-18755-4.

Park, G.-H., Noh, H., Shao, Z., Ni, P., Qin, Y., Liu, D., Beaudreault, C.P., Park, J.S., Abani, C.P., Park, J.M., et al. (2020). Activated microglia cause metabolic disruptions in developmental cortical interneurons that persist in interneurons from individuals with schizophrenia. Nat. Neurosci. *23*, 1352–1364. https://doi.org/10.1038/s41593-020-00724-1.

Park, J., Wetzel, I., Marriott, I., Dréau, D., D'Avanzo, C., Kim, D.Y., Tanzi, R.E., and Cho, H. (2018). A 3D human triculture system modeling neurodegeneration and neuroinflammation in Alzheimer's disease. Nat. Neurosci. *21*, 941–951. https://doi.org/10.1038/s41593-018-0175-4.

Paşca, S.P. (2024). Constructing human neural circuits in living systems by transplantation. Cell *187*, 8–13. https://doi.org/10.1016/j.cell.2023.12.008.

Paşca, S.P., Portmann, T., Voineagu, I., Yazawa, M., Shcheglovitov, A., Paşca, A.M., Cord, B., Palmer, T.D., Chikahisa, S., Nishino, S., et al. (2011). Using iPSC-derived neurons to uncover cellular phenotypes associated with Timothy syndrome. Nat. Med. *17*, 1657–1662. https://doi.org/10.1038/nm.2576.

Patkee, P.A., Baburamani, A.A., Kyriakopoulou, V., Davidson, A., Avini, E., Dimitrova, R., Allsop, J., Hughes, E., Kangas, J., McAlonan, G., and Rutherford, M.A. (2020). Early alterations in cortical and cerebellar regional brain growth in Down Syndrome: An in vivo fetal and neonatal MRI assessment. Neuroimage. Clin. *25*, 102139. https://doi.org/10.1016/j.nicl.2019.102139.

Penney, J., Ralvenius, W.T., and Tsai, L.-H. (2020). Modeling Alzheimer's disease with iPSC-derived brain cells. Mol. Psychiatry *25*, 148–167. https://doi.org/10.1038/s41380-019-0468-3.

Perez, S.M., and Lodge, D.J. (2013). Hippocampal interneuron transplants reverse aberrant dopamine system function and behavior in a rodent model of schizophrenia. Mol. Psychiatry *18*, 1193–1198. https://doi.org/10.1038/mp.2013.111.

Perez, S.M., Shah, A., Asher, A., and Lodge, D.J. (2013). Hippocampal deep brain stimulation reverses physiological and behavioural deficits in a rodent model of schizophrenia. Int. J. Neuropsychopharmacol. *16*, 1331–1339. https://doi.org/10.1017/S1461145712001344.

Piao, J., Zabierowski, S., Dubose, B.N., Hill, E.J., Navare, M., Claros, N., Rosen, S., Ramnarine, K., Horn, C., Fredrickson, C., et al. (2021). Preclinical Efficacy and Safety of a Human Embryonic Stem Cell-Derived Midbrain Dopamine Progenitor Product, MSK-DA01.

Cell Stem Cell *28*, 217–229.e7. https://doi.org/10.1016/j.stem.2021.01.004.

Pickett, E.K., Herrmann, A.G., McQueen, J., Abt, K., Dando, O., Tulloch, J., Jain, P., Dunnett, S., Sohrabi, S., Fjeldstad, M.P., et al. (2019). Amyloid Beta and Tau Cooperate to Cause Reversible Behavioral and Transcriptional Deficits in a Model of Alzheimer's Disease. Cell Rep. *29*, 3592–3604.e5. https://doi.org/10.1016/j.celrep.2019.11.044.

Pitkänen, A., Nissinen, J., Nairismägi, J., Lukasiuk, K., Gröhn, O.H.J., Miettinen, R., and Kauppinen, R. (2002). Progression of neuronal damage after status epilepticus and during spontaneous seizures in a rat model of temporal lobe epilepsy. In Progress in Brain Research (Elsevier), pp. 67–83.

Polymeropoulos, M.H., Lavedan, C., Leroy, E., Ide, S.E., Dehejia, A., Dutra, A., Pike, B., Root, H., Rubenstein, J., Boyer, R., et al. (1997). Mutation in the alpha-synuclein gene identified in families with Parkinson's disease. Science *276*, 2045–2047. https://doi.org/10.1126/science.276.5321.2045.

Ponroy Bally, B., Farmer, W.T., Jones, E.V., Jessa, S., Kacerovsky, J.B., Mayran, A., Peng, H., Lefebvre, J.L., Drouin, J., Hayer, A., et al. (2020). Human iPSC-derived Down syndrome astrocytes display genome-wide perturbations in gene expression, an altered adhesion profile, and increased cellular dynamics. Hum. Mol. Genet. *29*, 785–802. https://doi.org/10.1093/hmg/ddaa003.

Preman, P., TCW, J., Calafate, S., Snellinx, A., Alfonso-Triguero, M., Corthout, N., Munck, S., Thal, D.R., Goate, A.M., De Strooper, B., and Arranz, A.M. (2021). Human iPSC-derived astrocytes transplanted into the mouse brain undergo morphological changes in response to amyloid-β plaques. Mol. Neurodegener. *16*, 68. https://doi.org/10.1186/s13024-021-00487-8.

Pueschel, S.M., Louis, S., and McKnight, P. (1991). Seizure disorders in Down syndrome. Arch. Neurol. *48*, 318–320. https://doi.org/10.1001/archneur.1991.00530150088024.

Purdy, I.B., Singh, N., Brown, W.L., Vangala, S., and Devaskar, U.P. (2014). Revisiting early hypothyroidism screening in infants with Down syndrome. J. Perinatol. *34*, 936–940. https://doi.org/10.1038/jp.2014.116.

Putnam, T.J., and Merritt, H.H. (1937). Experimental Determination of the Anticonvulsant Properties of Some Phenyl Derivatives. Science *85*, 525–526. https://doi.org/10.1126/science.

Raunio, A., Kaivola, K., Tuimala, J., Kero, M., Oinas, M., Polvikoski, T., Paetau, A., Tienari, P.J., and Myllykangas, L. (2019). Lewy-related pathology exhibits two anatomically and genetically distinct progression patterns: a population-based study of Finns aged 85. Acta Neuropathol. *138*, 771–782. https://doi.org/10.1007/s00401-019-02071-3.

Raz, N., Torres, I.J., Briggs, S.D., Spencer, W.D., Thornton, A.E., Loken, W.J., Gunning, F.M., McQuain, J.D., Driesen, N.R., and Acker, J.D. (1995). Selective neuroanatomic abnormalities in Down's syndrome and their cognitive correlates: evidence from MRI morphometry. Neurology *45*, 356–366. https://doi.org/10.1212/wnl.45.2.356.

Real, R., Peter, M., Trabalza, A., Khan, S., Smith, M.A., Dopp, J., Barnes, S.J., Momoh, A., Strano, A., Volpi, E., et al. (2018). In vivo modeling of human neuron dynamics and Down syndrome. Science *362*, eaau1810. https://doi.org/10.1126/science.aau1810.

Reeves, R.H., Irving, N.G., Moran, T.H., Wohn, A., Kitt, C., Sisodia, S.S., Schmidt, C., Bronson, R.T., and Davisson, M.T. (1995). A mouse model for Down syndrome exhibits learning and behaviour deficits. Nat. Genet. *11*, 177–184. https://doi.org/10.1038/ng1095-177.

Revah, O., Gore, F., Kelley, K.W., Andersen, J., Sakai, N., Chen, X., Li, M.-Y., Birey, F., Yang, X., Saw, N.L., et al. (2022). Maturation and circuit integration of transplanted human cortical organoids. Nature *610*, 319–326. https://doi.org/10.1038/s41586-022-05277-w.

Riban, V., Bouilleret, V., Pham-Lê, B.T., Fritschy, J.-M., Marescaux, C., and Depaulis, A. (2002). Evolution of hippocampal epileptic activity during the development of hippocampal sclerosis in a mouse model of temporal lobe epilepsy. Neuroscience *112*, 101–111. https://doi.org/10.1016/s0306-4522(02)00064-7.

Richards, R.K., and Everett, G.M. (1946). Tridione: a new anticonvulsant drug. J. Lab. Clin. Med. *31*, 1330–1336.

Roberson, E.D., Scearce-Levie, K., Palop, J.J., Yan, F., Cheng, I.H., Wu, T., Gerstein, H., Yu, G.-Q., and Mucke, L. (2007). Reducing endogenous tau ameliorates amyloid beta-induced deficits in an Alzheimer's disease mouse model. Science *316*, 750–754. https://doi.org/10.1126/science.1141736.

Rodriques, S.G., Stickels, R.R., Goeva, A., Martin, C.A., Murray, E., Vanderburg, C.R., Welch, J., Chen, L.M., Chen, F., and Macosko, E.Z. (2019). Slide-seq: A scalable technology for measuring genome-wide expression at high spatial resolution. Science *363*, 1463–1467. https://doi.org/10.1126/science.aaw1219.

Roizen, N.J., Mets, M.B., and Blondis, T.A. (1994). Ophthalmic disorders in children with Down syndrome. Dev. Med. Child Neurol. *36*, 594–600. https://doi.org/10.1111/j.1469-8749.1994.tb11896.x.

Rousseaux, M.W.C., Marcogliese, P.C., Qu, D., Hewitt, S.J., Seang, S., Kim, R.H., Slack, R.S., Schlossmacher, M.G., Lagace, D.C., Mak, T.W., and Park, D.S. (2012). Progressive dopaminergic cell loss with unilateral-to-bilateral progression in a genetic model of Parkinson disease. Proc. Natl. Acad. Sci. USA *109*, 15918–15923. https://doi.org/10.1073/pnas.1205102109.

Roy, N.S., Cleren, C., Singh, S.K., Yang, L., Beal, M.F., and Goldman, S.A. (2006). Functional engraftment of human ES cell-derived dopaminergic neurons enriched by coculture with telomerase-immortalized midbrain astrocytes. Nat. Med. *12*, 1259–1268. https://doi.org/10.1038/nm1495.

Rubinstein, M., Westenbroek, R.E., Yu, F.H., Jones, C.J., Scheuer, T., and Catterall, W.A. (2015). Genetic background modulates impaired excitability of inhibitory neurons in a mouse model of Dravet syndrome. Neurobiol. Dis. *73*, 106–117. https://doi.org/10.1016/j.nbd.2014.09.017.

Ryan, S.D., Dolatabadi, N., Chan, S.F., Zhang, X., Akhtar, M.W., Parker, J., Soldner, F., Sunico, C.R., Nagar, S., Talantova, M., et al. (2013). Isogenic Human iPSC Parkinson's Model Shows Nitrosative Stress-Induced Dysfunction in MEF2-PGC1α Transcription. Cell *155*, 1351–1364. https://doi.org/10.1016/j.cell.2013.11.009.

Sackmann, C., and Hallbeck, M. (2020). Oligomeric amyloid-β induces early and widespread changes to the proteome in human

iPSC-derived neurons. Sci. Rep. *10*, 6538. https://doi.org/10.1038/s41598-020-63398-6.

Sago, H., Carlson, E.J., Smith, D.J., Kilbridge, J., Rubin, E.M., Mobley, W.C., Epstein, C.J., and Huang, T.-T. (1998). Ts1Cje, a partial trisomy 16 mouse model for Down syndrome, exhibits learning and behavioral abnormalities. Proc. Natl. Acad. Sci. USA *95*, 6256–6261. https://doi.org/10.1073/pnas.95.11.6256.

Saito, Y., Kawashima, A., Ruberu, N.N., Fujiwara, H., Koyama, S., Sawabe, M., Arai, T., Nagura, H., Yamanouchi, H., Hasegawa, M., et al. (2003). Accumulation of phosphorylated alpha-synuclein in aging human brain. J. Neuropathol. Exp. Neurol. *62*, 644–654. https://doi.org/10.1093/jnen/62.6.644.

Samarasinghe, R.A., Miranda, O.A., Buth, J.E., Mitchell, S., Ferando, I., Watanabe, M., Allison, T.F., Kurdian, A., Fotion, N.N., Gandal, M.J., et al. (2021). Identification of neural oscillations and epileptiform changes in human brain organoids. Nat. Neurosci. *24*, 1488–1500. https://doi.org/10.1038/s41593-021-00906-5.

Schafer, S.T., Mansour, A.A., Schlachetzki, J.C.M., Pena, M., Ghassemzadeh, S., Mitchell, L., Mar, A., Quang, D., Stumpf, S., Ortiz, I.S., et al. (2023). An in vivo neuroimmune organoid model to study human microglia phenotypes. Cell *186*, 2111–2126.e20. https://doi.org/10.1016/j.cell.2023.04.022.

Schauwecker, P.E. (2011). The relevance of individual genetic background and its role in animal models of epilepsy. Epilepsy Res. *97*, 1–11. https://doi.org/10.1016/j.eplepsyres.2011.09.005.

Scheffer, I.E., Berkovic, S., Capovilla, G., Connolly, M.B., French, J., Guilhoto, L., Hirsch, E., Jain, S., Mathern, G.W., Moshé, S.L., et al. (2017). ILAE classification of the epilepsies: Position paper of the ILAE Commission for Classification and Terminology. Epilepsia *58*, 512–521. https://doi.org/10.1111/epi.13709.

Schrode, N., Ho, S.-M., Yamamuro, K., Dobbyn, A., Huckins, L., Matos, M.R., Cheng, E., Deans, P.J.M., Flaherty, E., Barretto, N., et al. (2019). Synergistic effects of common schizophrenia risk variants. Nat. Genet. *51*, 1475–1485. https://doi.org/10.1038/s41588-019-0497-5.

Schweitzer, J.S., Song, B., Herrington, T.M., Park, T.-Y., Lee, N., Ko, S., Jeon, J., Cha, Y., Kim, K., Li, Q., et al. (2020). Personalized iPSC-Derived Dopamine Progenitor Cells for Parkinson's Disease. N. Engl. J. Med. *382*, 1926–1932. https://doi.org/10.1056/NEJMoa1915872.

Schwob, J.E., Fuller, T., Price, J.L., and Olney, J.W. (1980). Widespread patterns of neuronal damage following systemic or intracerebral injections of kainic acid: A histological study. Neuroscience *5*, 991–1014. https://doi.org/10.1016/0306-4522(80)90181-5.

Sellgren, C.M., Gracias, J., Watmuff, B., Biag, J.D., Thanos, J.M., Whittredge, P.B., Fu, T., Worringer, K., Brown, H.E., Wang, J., et al. (2019). Increased synapse elimination by microglia in schizophrenia patient-derived models of synaptic pruning. Nat. Neurosci. *22*, 374–385. https://doi.org/10.1038/s41593-018-0334-7.

Seo, H., and Isacson, O. (2005). Abnormal APP, cholinergic and cognitive function in Ts65Dn Down's model mice. Exp. Neurol. *193*, 469–480. https://doi.org/10.1016/j.expneurol.2004.11.017.

Shi, Y., Kirwan, P., Smith, J., MacLean, G., Orkin, S.H., and Livesey, F.J. (2012). A Human Stem Cell Model of Early Alzheimer's Disease Pathology in Down Syndrome. Sci. Transl. Med. *4*, 124ra29. https://doi.org/10.1126/scitranslmed.3003771.

Shin, J.-H., Ko, H.S., Kang, H., Lee, Y., Lee, Y.-I., Pletinkova, O., Troconso, J.C., Dawson, V.L., and Dawson, T.M. (2011). PARIS (ZNF746) repression of PGC-1α contributes to neurodegeneration in Parkinson's disease. Cell *144*, 689–702. https://doi.org/10.1016/j.cell.2011.02.010.

Sidoryk-Wegrzynowicz, M., Gerber, Y.N., Ries, M., Sastre, M., Tolkovsky, A.M., and Spillantini, M.G. (2017). Astrocytes in mouse models of tauopathies acquire early deficits and lose neurosupportive functions. Acta Neuropathol. Commun. *5*, 89. https://doi.org/10.1186/s40478-017-0478-9.

Silbereis, J.C., Pochareddy, S., Zhu, Y., Li, M., and Sestan, N. (2016). The Cellular and Molecular Landscapes of the Developing Human Central Nervous System. Neuron *89*, 248–268. https://doi.org/10.1016/j.neuron.2015.12.008.

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., et al. (2019). Modeling Parkinson's disease in midbrain-like organoids. NPJ Parkinsons Dis. *5*, 5. https://doi.org/10.1038/s41531-019-0078-4.

Soldner, F., Stelzer, Y., Shivalila, C.S., Abraham, B.J., Latourelle, J.C., Barrasa, M.I., Goldmann, J., Myers, R.H., Young, R.A., and Jaenisch, R. (2016). Parkinson-associated risk variant in distal enhancer of α-synuclein modulates target gene expression. Nature *533*, 95–99. https://doi.org/10.1038/nature17939.

Sommer, W. (1880). Erkrankung des Ammonshorns als aetiologisches Moment der Epilepsie. Archiv f. Psychiatrie *10*, 631–675. https://doi.org/10.1007/BF02224538.

Song, B., Cha, Y., Ko, S., Jeon, J., Lee, N., Seo, H., Park, K.-J., Lee, I.-H., Lopes, C., Feitosa, M., et al. (2020). Human autologous iPSC-derived dopaminergic progenitors restore motor function in Parkinson's disease models. J. Clin. Invest. *130*, 904–920. https://doi.org/10.1172/JCI130767.

Sonninen, T.-M., Hämäläinen, R.H., Koskuvi, M., Oksanen, M., Shakirzyanova, A., Wojciechowski, S., Puttonen, K., Naumenko, N., Goldsteins, G., Laham-Karam, N., et al. (2020). Metabolic alterations in Parkinson's disease astrocytes. Sci. Rep. *10*, 14474. https://doi.org/10.1038/s41598-020-71329-8.

Soreq, L., UK Brain Expression Consortium, North American Brain Expression Consortium, Rose, J., Soreq, E., Hardy, J., Trabzuni, D., Cookson, M.R., Smith, C., Ryten, M., et al. (2017). Major Shifts in Glial Regional Identity Are a Transcriptional Hallmark of Human Brain Aging. Cell Rep. *18*, 557–570. https://doi.org/10.1016/j.celrep.2016.12.011.

Spillantini, M.G., Schmidt, M.L., Lee, V.M., Trojanowski, J.Q., Jakes, R., and Goedert, M. (1997). α-Synuclein in Lewy bodies. Nature *388*, 839–840. https://doi.org/10.1038/42166.

Sproul, A.A., Jacob, S., Pre, D., Kim, S.H., Nestor, M.W., Navarro-Sobrino, M., Santa-Maria, I., Zimmer, M., Aubry, S., Steele, J.W., et al. (2014). Characterization and molecular profiling of PSEN1 familial Alzheimer's disease iPSC-derived neural progenitors. PLoS One *9*, e84547. https://doi.org/10.1371/journal.pone.0084547.

St Clair, D., Xu, M., Wang, P., Yu, Y., Fang, Y., Zhang, F., Zheng, X., Gu, N., Feng, G., Sham, P., and He, L. (2005). Rates of adult

schizophrenia following prenatal exposure to the Chinese famine of 1959-1961. JAMA *294*, 557–562. https://doi.org/10.1001/jama.294.5.557.

Stafstrom, C.E., and Carmant, L. (2015). Seizures and Epilepsy: An Overview for Neuroscientists. Cold Spring Harb. Perspect. Med. *5*, a022426. https://doi.org/10.1101/cshperspect.a022426.

Stagni, F., Giacomini, A., Emili, M., Uguagliati, B., Bonasoni, M.P., Bartesaghi, R., and Guidi, S. (2020). Neuroanatomical alterations in higher-order thalamic nuclei of fetuses with Down syndrome. Clin. Neurol. Neurosurg. *194*, 105870. https://doi.org/10.1016/j.clineuro.2020.105870.

Ståhl, P.L., Salmén, F., Vickovic, S., Lundmark, A., Navarro, J.F., Magnusson, J., Giacomello, S., Asp, M., Westholm, J.O., Huss, M., et al. (2016). Visualization and analysis of gene expression in tissue sections by spatial transcriptomics. Science *353*, 78–82. https://doi.org/10.1126/science.aaf2403.

Stan, A.D., Ghose, S., Gao, X.-M., Roberts, R.C., Lewis-Amezcua, K., Hatanpaa, K.J., and Tamminga, C.A. (2006). Human Postmortem Tissue: What Quality Markers Matter? Brain Res. *1123*, 1–11. https://doi.org/10.1016/j.brainres.2006.09.025.

Steinbeck, J.A., Choi, S.J., Mrejeru, A., Ganat, Y., Deisseroth, K., Sulzer, D., Mosharov, E.V., and Studer, L. (2015). Optogenetics enables functional analysis of human embryonic stem cell–derived grafts in a Parkinson's disease model. Nat. Biotechnol. *33*, 204–209. https://doi.org/10.1038/nbt.3124.

Steinberg, D.J., Repudi, S., Saleem, A., Kustanovich, I., Viukov, S., Abudiab, B., Banne, E., Mahajnah, M., Hanna, J.H., Stern, S., et al. (2021). Modeling genetic epileptic encephalopathies using brain organoids. EMBO Mol. Med. *13*, e13610. https://doi.org/10.15252/emmm.202013610.

Su, C.-J., Feng, Y., Liu, T.-T., Liu, X., Bao, J.-J., Shi, A.-M., Hu, D.-M., Liu, T., and 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.

Suetsugu, M., and Mehraein, P. (1980). Spine distribution along the apical dendrites of the pyramidal neurons in Down's syndrome. A quantitative Golgi study. Acta Neuropathol. *50*, 207–210. https://doi.org/10.1007/BF00688755.

Sun, L., Shen, R., Agnihotri, S.K., Chen, Y., Huang, Z., and Büeler, H. (2018). Lack of PINK1 alters glia innate immune responses and enhances inflammation-induced, nitric oxide-mediated neuron death. Sci. Rep. *8*, 383. https://doi.org/10.1038/s41598-017-18786-w.

Sun, Y., Paşca, S.P., Portmann, T., Goold, C., Worringer, K.A., Guan, W., Chan, K.C., Gai, H., Vogt, D., Chen, Y.-J.J., et al. (2016). A deleterious Nav1.1 mutation selectively impairs telencephalic inhibitory neurons derived from Dravet Syndrome patients. Elife *5*, e13073. https://doi.org/10.7554/eLife.13073.

Susser, E.S., and Lin, S.P. (1992). Schizophrenia after prenatal exposure to the Dutch Hunger Winter of 1944-1945. Arch. Gen. Psychiatry *49*, 983–988. https://doi.org/10.1001/archpsyc.1992.01820120071010.

Swinyard, E.A. (1949). Laboratory assay of clinically effective antiepileptic drugs. This investigation was supported from the Division of Research Grants and Fellowships of the National Institute of Health, U. S. Public Health Service. J. Am. Pharm. Assoc. Am. Pharm. Assoc. *38*, 201–204. https://doi.org/10.1002/jps.3030380409.

Takahashi, K., and Yamanaka, S. (2006). Induction of Pluripotent Stem Cells from Mouse Embryonic and Adult Fibroblast Cultures by Defined Factors. Cell *126*, 663–676. https://doi.org/10.1016/j.cell.2006.07.024.

Takashima, A., Noguchi, K., Sato, K., Hoshino, T., and Imahori, K. (1993). Tau protein kinase I is essential for amyloid beta-protein-induced neurotoxicity. Proc. Natl. Acad. Sci. USA *90*, 7789–7793. https://doi.org/10.1073/pnas.90.16.7789.

Takashima, S., Becker, L.E., Armstrong, D.L., and Chan, F. (1981). Abnormal neuronal development in the visual cortex of the human fetus and infant with down's syndrome. A quantitative and qualitative Golgi study. Brain Res. *225*, 1–21. https://doi.org/10.1016/0006-8993(81)90314-0.

Tandberg, E., Larsen, J.P., Aarsland, D., and Cummings, J.L. (1996). The occurrence of depression in Parkinson's disease. A community-based study. Arch. Neurol. *53*, 175–179. https://doi.org/10.1001/archneur.1996.00550020087019.

Tang, X., Kim, J., Zhou, L., Wengert, E., Zhang, L., Wu, Z., Carromeu, C., Muotri, A.R., Marchetto, M.C.N., Gage, F.H., and Chen, G. (2016). KCC2 rescues functional deficits in human neurons derived from patients with Rett syndrome. Proc. Natl. Acad. Sci. USA *113*, 751–756. https://doi.org/10.1073/pnas.1524013113.

Tang, X.-Y., Xu, L., Wang, J., Hong, Y., Wang, Y., Zhu, Q., Wang, D., Zhang, X.-Y., Liu, C.-Y., Fang, K.-H., et al. (2021). DSCAM/PAK1 pathway suppression reverses neurogenesis deficits in iPSC-derived cerebral organoids from patients with Down syndrome. J. Clin. Invest. *131*, 135763. https://doi.org/10.1172/JCI135763.

Toda, M., and Abi-Dargham, A. (2007). Dopamine hypothesis of schizophrenia: making sense of it all. Curr. Psychiatry Rep. *9*, 329–336. https://doi.org/10.1007/s11920-007-0041-7.

Toman, J.E.P., Swinyard, E.A., and Goodman, L.S. (1946). PROPERTIES OF MAXIMAL SEIZURES, AND THEIR ALTERATION BY ANTICONVULSANT DRUGS AND OTHER AGENTS. J. Neurophysiol. *9*, 231–239. https://doi.org/10.1152/jn.1946.9.3.231.

Uchida, T., Lossin, C., Ihara, Y., Deshimaru, M., Yanagawa, Y., Koyama, S., and Hirose, S. (2017). Abnormal γ-aminobutyric acid neurotransmission in a *Kcnq2* model of early onset epilepsy. Epilepsia *58*, 1430–1439. https://doi.org/10.1111/epi.13807.

Upadhya, D., Hattiangady, B., Shetty, G.A., Zanirati, G., Kodali, M., and Shetty, A.K. (2016). Neural Stem Cell or Human Induced Pluripotent Stem Cell-derived GABA-ergic Progenitor Cell Grafting in an Animal Model of Chronic Temporal Lobe Epilepsy. Curr. Protoc. Stem Cell Biol. *38*, 1–2D.7.47. https://doi.org/10.1002/cpsc.9.

Upadhya, D., Hattiangady, B., Castro, O.W., Shuai, B., Kodali, M., Attaluri, S., Bates, A., Dong, Y., Zhang, S.-C., Prockop, D.J., and Shetty, A.K. (2019). Human induced pluripotent stem cell-derived MGE cell grafting after status epilepticus attenuates chronic epilepsy and comorbidities via synaptic integration. Proc. Natl. Acad. Sci. USA *116*, 287–296. https://doi.org/10.1073/pnas.1814185115.

Van Arman, C.G. (1951). Amino acids and amines as precursors of epinephrine. Am. J. Physiol. *164*, 476–479. https://doi.org/10.1152/ajplegacy.1951.164.2.476.

Vandamme, T.F. (2014). Use of rodents as models of human diseases. J. Pharm. BioAllied Sci. *6*, 2–9. https://doi.org/10.4103/0975-7406.124301.

Vergnes, M., Marescaux, C., Micheletti, G., Reis, J., Depaulis, A., Rumbach, L., and Warter, J.M. (1982). Spontaneous paroxysmal electroclinical patterns in rat: A model of generalized non-convulsive epilepsy. Neurosci. Lett. *33*, 97–101. https://doi.org/10.1016/0304-3940(82)90136-7.

Vila Verde, D., Zimmer, T., Cattalini, A., Pereira, M.F., van Vliet, E.A., Testa, G., Gnatkovsky, V., Aronica, E., and de Curtis, M. (2021). Seizure activity and brain damage in a model of focal non-convulsive status epilepticus. Neuropathol. Appl. Neurobiol. *47*, 679–693. https://doi.org/10.1111/nan.12693.

Wadman, M. (2023). FDA no longer has to require animal testing for new drugs. Science *379*, 127–128. https://doi.org/10.1126/science.adg6276.

Waldau, B., Hattiangady, B., Kuruba, R., and Shetty, A.K. (2010). Medial ganglionic eminence-derived neural stem cell grafts ease spontaneous seizures and restore GDNF expression in a rat model of chronic temporal lobe epilepsy. Stem Cell. *28*, 1153–1164. https://doi.org/10.1002/stem.446.

Waloschková, E., Gonzalez-Ramos, A., Mikroulis, A., Kudláček, J., Andersson, M., Ledri, M., and Kokaia, M. (2021). Human Stem Cell-Derived GABAergic Interneurons Establish Efferent Synapses onto Host Neurons in Rat Epileptic Hippocampus and Inhibit Spontaneous Recurrent Seizures. IJMS *22*, 13243. https://doi.org/10.3390/ijms222413243.

Walsh, D.M., Klyubin, I., Fadeeva, J.V., Cullen, W.K., Anwyl, R., Wolfe, M.S., Rowan, M.J., and Selkoe, D.J. (2002). Naturally secreted oligomers of amyloid beta protein potently inhibit hippocampal long-term potentiation in vivo. Nature *416*, 535–539. https://doi.org/10.1038/416535a.

Wang, C., Najm, R., Xu, Q., Jeong, D.-E., Walker, D., Balestra, M.E., Yoon, S.Y., Yuan, H., Li, G., Miller, Z.A., et al. (2018). Gain of toxic apolipoprotein E4 effects in human iPSC-derived neurons is ameliorated by a small-molecule structure corrector. Nat. Med. *24*, 647–657. https://doi.org/10.1038/s41591-018-0004-z.

Wang, Y., Greenwood, J.S.F., Calcagnotto, M.E., Kirsch, H.E., Barbaro, N.M., and Baraban, S.C. (2007). Neocortical hyperexcitability in a human case of tuberous sclerosis complex and mice lacking neuronal expression of *TSC1*. Ann. Neurol. *61*, 139–152. https://doi.org/10.1002/ana.21058.

Wang, Y., Cella, M., Mallinson, K., Ulrich, J.D., Young, K.L., Robinette, M.L., Gilfillan, S., Krishnan, G.M., Sudhakar, S., Zinselmeyer, B.H., et al. (2015). TREM2 lipid sensing sustains the microglial response in an Alzheimer's disease model. Cell *160*, 1061–1071. https://doi.org/10.1016/j.cell.2015.01.049.

Wax, P.M. (1995). Elixirs, diluents, and the passage of the 1938 Federal Food, Drug and Cosmetic Act. Ann. Intern. Med. *122*, 456–461. https://doi.org/10.7326/0003-4819-122-6-199503150-00009.

Weick, J.P., Held, D.L., Bonadurer, G.F., Doers, M.E., Liu, Y., Maguire, C., Clark, A., Knackert, J.A., Molinarolo, K., Musser, M., et al. (2013). Deficits in human trisomy 21 iPSCs and neurons. Proc. Natl. Acad. Sci. USA *110*, 9962–9967. https://doi.org/10.1073/pnas.1216575110.

Wilkins, R.H., and Brody, I.A. (1971). Down's syndrome. Arch. Neurol. *25*, 88–90. https://doi.org/10.1001/archneur.1971.00490010098013.

Winden, K.D., Sundberg, M., Yang, C., Wafa, S.M.A., Dwyer, S., Chen, P.-F., Buttermore, E.D., and Sahin, M. (2019). Biallelic Mutations in *TSC2* Lead to Abnormalities Associated with Cortical Tubers in Human iPSC-Derived Neurons. J. Neurosci. *39*, 9294–9305. https://doi.org/10.1523/JNEUROSCI.0642-19.2019.

Windrem, M.S., Osipovitch, M., Liu, Z., Bates, J., Chandler-Militello, D., Zou, L., Munir, J., Schanz, S., McCoy, K., Miller, R.H., et al. (2017). Human iPSC Glial Mouse Chimeras Reveal Glial Contributions to Schizophrenia. Cell Stem Cell *21*, 195–208.e6. https://doi.org/10.1016/j.stem.2017.06.012.

Wisniewski, K.E. (1990). Down syndrome children often have brain with maturation delay, retardation of growth, and cortical dysgenesis. Am. J. Med. Genet. Suppl. *7*, 274–281. https://doi.org/10.1002/ajmg.1320370755.

Wu, C.-I., Vinton, E.A., Pearse, R.V., Heo, K., Aylward, A.J., Hsieh, Y.-C., Bi, Y., Adeleye, S., Fancher, S., Duong, D.M., et al. (2022). APP and DYRK1A regulate axonal and synaptic vesicle protein networks and mediate Alzheimer's pathology in trisomy 21 neurons. Mol. Psychiatry *27*, 1970–1989. https://doi.org/10.1038/s41380-022-01454-5.

Wulansari, N., Darsono, W.H.W., Woo, H.-J., Chang, M.-Y., Kim, J., Bae, E.-J., Sun, W., Lee, J.-H., Cho, I.-J., Shin, H., et al. (2021). Neurodevelopmental defects and neurodegenerative phenotypes in human brain organoids carrying Parkinson's disease-linked DNAJC6 mutations. Sci. Adv. *7*, eabb1540. https://doi.org/10.1126/sciadv.abb1540.

Xiong, M., Tao, Y., Gao, Q., Feng, B., Yan, W., Zhou, Y., Kotsonis, T.A., Yuan, T., You, Z., Wu, Z., et al. (2021). Human Stem Cell-Derived Neurons Repair Circuits and Restore Neural Function. Cell Stem Cell *28*, 112–126.e6. https://doi.org/10.1016/j.stem.2020.08.014.

Xu, K., Liu, F., Xu, W., Liu, J., Chen, S., and Wu, G. (2019a). Transplanting GABAergic Neurons Differentiated from Neural Stem Cells into Hippocampus Inhibits Seizures and Epileptiform Discharges in Pilocarpine-Induced Temporal Lobe Epilepsy Model. World Neurosurg. *128*, e1–e11. https://doi.org/10.1016/j.wneu.2019.01.245.

Xu, L., Huo, H.-Q., Lu, K.-Q., Tang, X.-Y., Hong, Y., Han, X., Fu, Z.-X., Fang, K.-H., Xu, M., Guo, X., and Liu, Y. (2022). Abnormal mitochondria in Down syndrome iPSC-derived GABAergic interneurons and organoids. Biochim. Biophys. Acta, Mol. Basis Dis. *1868*, 166388. https://doi.org/10.1016/j.bbadis.2022.166388.

Xu, M., Zhang, L., Liu, G., Jiang, N., Zhou, W., and Zhang, Y. (2019b). Pathological Changes in Alzheimer's Disease Analyzed Using Induced Pluripotent Stem Cell-Derived Human Microglia-Like Cells. J. Alzheimers Dis. *67*, 357–368. https://doi.org/10.3233/JAD-180722.

Xu, R., Brawner, A.T., Li, S., Liu, J.-J., Kim, H., Xue, H., Pang, Z.P., Kim, W.-Y., Hart, R.P., Liu, Y., and Jiang, P. (2019c). OLIG2 Drives Abnormal Neurodevelopmental Phenotypes in Human iPSC-Based Organoid and Chimeric Mouse Models of Down Syndrome. Cell Stem Cell *24*, 908–926.e8. https://doi.org/10.1016/j.stem.2019.04.014.

Yagi, T., Ito, D., Okada, Y., Akamatsu, W., Nihei, Y., Yoshizaki, T., Yamanaka, S., Okano, H., and Suzuki, N. (2011). Modeling familial Alzheimer's disease with induced pluripotent stem cells. Hum. Mol. Genet. *20*, 4530–4539. https://doi.org/10.1093/hmg/ddr394.

Yahr, M.D., Duvoisin, R.C., Schear, M.J., Barrett, R.E., and Hoehn, M.M. (1969). Treatment of parkinsonism with levodopa. Arch. Neurol. *21*, 343–354. https://doi.org/10.1001/archneur.1969.00480160015001.

Yamaguchi, H., Hirai, S., Morimatsu, M., Shoji, M., and Ihara, Y. (1988). A variety of cerebral amyloid deposits in the brains of the Alzheimer-type dementia demonstrated by beta protein immunostaining. Acta Neuropathol. *76*, 541–549. https://doi.org/10.1007/BF00689591.

Yoshiyama, Y., Higuchi, M., Zhang, B., Huang, S.-M., Iwata, N., Saido, T.C., Maeda, J., Suhara, T., Trojanowski, J.Q., and Lee, V.M.-Y. (2007). Synapse loss and microglial activation precede tangles in a P301S tauopathy mouse model. Neuron *53*, 337–351. https://doi.org/10.1016/j.neuron.2007.01.010.

Yu, F.H., Mantegazza, M., Westenbroek, R.E., Robbins, C.A., Kalume, F., Burton, K.A., Spain, W.J., McKnight, G.S., Scheuer, T., and Catterall, W.A. (2006). Reduced sodium current in GABAergic interneurons in a mouse model of severe myoclonic epilepsy in infancy. Nat. Neurosci. *9*, 1142–1149. https://doi.org/10.1038/nn1754.

Yu, T., Li, Z., Jia, Z., Clapcote, S.J., Liu, C., Li, S., Asrar, S., Pao, A., Chen, R., Fan, N., et al. (2010). A mouse model of Down syndrome trisomic for all human chromosome 21 syntenic regions. Hum. Mol. Genet. *19*, 2780–2791. https://doi.org/10.1093/hmg/ddq179.

Zdaniuk, G., Wierzba-Bobrowicz, T., Szpak, G.M., and Stępień, T. (2011). Astroglia disturbances during development of the central nervous system in fetuses with Down's syndrome. Folia Neuropathol. *49*, 109–114.

Zhang, Y., Pak, C., Han, Y., Ahlenius, H., Zhang, Z., Chanda, S., Marro, S., Patzke, C., Acuna, C., Covy, J., et al. (2013). Rapid Single-Step Induction of Functional Neurons from Human Pluripotent Stem Cells. Neuron *78*, 785–798. https://doi.org/10.1016/j.neuron.2013.05.029.

Zhao, J., Fu, Y., Yamazaki, Y., Ren, Y., Davis, M.D., Liu, C.-C., Lu, W., Wang, X., Chen, K., Cherukuri, Y., et al. (2020). APOE4 exacerbates synapse loss and neurodegeneration in Alzheimer's disease patient iPSC-derived cerebral organoids. Nat. Commun. *11*, 5540. https://doi.org/10.1038/s41467-020-19264-0.

Zhu, Q., Mishra, A., Park, J.S., Liu, D., Le, D.T., Gonzalez, S.Z., Anderson-Crannage, M., Park, J.M., Park, G.-H., Tarbay, L., et al. (2023). Human cortical interneurons optimized for grafting specifically integrate, abort seizures, and display prolonged efficacy without over-inhibition. Neuron *111*, 807–823.e7. https://doi.org/10.1016/j.neuron.2022.12.014.

Zucco, A.J., Pozzo, V.D., Afinogenova, A., Hart, R.P., Devinsky, O., and D'Arcangelo, G. (2018). Neural progenitors derived from Tuberous Sclerosis Complex patients exhibit attenuated PI3K/AKT signaling and delayed neuronal differentiation. Mol. Cell. Neurosci. *92*, 149–163. https://doi.org/10.1016/j.mcn.2018.08.004.