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Neurotropic Effects of SARS-CoV-2 Modeled by the Human Brain Organoids

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COVID-19, caused by SARS-CoV-2, is a socioeconomic burden, which exhibits respiratory illness along with unexpected neurological complications. Concerns have been raised about whether the observed neurological symptoms are due to direct effects on CNS or associated with the virus's systemic effect. Recent SARS-CoV-2 infection studies using human brain organoids revealed that SARS-CoV-2 targets human neurons. Human brain organoids are stem cell-derived reductionist experimental systems that have highlighted the neurotropic effects of SARS-CoV-2. Here, we summarize the neurotoxic effects of SARS-CoV-2 using brain organoids and comprehensively discuss how brain organoids could further improve our understanding when they are fine-tuned.

SARS-CoV-2 and its detrimental effects on the CNS

Severe acute respiratory syndrome corona virus 2 (SARS-CoV-2), which causes COVID-19, is a serious worldwide public health emergency of this century (Alabdulmonem et al., 2020). As of October 2020, at least 1.2 million human lives have succumbed to the disease, and several million have been infected. In terms of clinical symptoms, the COVID-19 disorders are similar to the previous SARS-CoV outbreak in 2003. These include respiratory illness with fever, dry cough, and dyspnea, indicating that the respiratory tract is the first target (Yuki et al., 2020; Hui et al., 2020; Subbarao and Mahanty, 2020). Although the observed neurological defects are not uncommon for coronaviruses, unexpectedly, a significant proportion of the COVID-19 patients exhibited severe neurological complications (Munhoz et al., 2020).

Even though the initial symptoms have been known to start with a headache, dizziness, and taste and smell impairment, the latter effects have been shown to be much more complicated and dependent on several factors, such as age group, sex, ethnicity, pre-existing conditions, and the severity of infection. The extended neurological symptoms include vision impairment, encephalitis, psychosis, neurocognitive impairment, dementia, cerebrovascular defects, ischemic strokes, and intracerebral hemorrhages (Mao et al., 2020; Ellul et al., 2020) (Table 1).

MRI-based screenings have also shown structural damage in the brains of patients recovering from COVID-19. This illustrates the potential long-term and persistent neurotoxic effects of SARS-CoV-2 in the CNS (Lu et al., 2020). Detecting the viral RNA in cerebrospinal fluid (CSF) of COVID-19 patients with neurological symptoms provided a strong hint that SARS-CoV-2 is associated with the CNS. Subsequently, a clinical report detected the presence of viral RNA in autopsy of brain samples (Edler et al., 2020; Xu et al., 2005). These clinical findings suggest that either the neurological symptoms are due to the direct effect of SARS-CoV-2 infecting the CNS or a systemic effect of the virus-mediated inflammation due to cytokine storm and aberrant immune response causing CNS insults. In the cytokine storm, the granulocytes are the key players that constitute the innate immune response, which is also well known because of their aggressive inflammatory reactions (Song et al., 2020; Tisoncik et al., 2012). Several studies have highlighted the deleterious effects of SARS-CoV-2 infections, which can compromise the host adaptive immune systems, such as B cells, T cells, and natural killer cells, at the same time triggering the innate immune response (Cao, 2020). These uncontrolled responses have been the cause of hyper inflammation and life-threatening conditions in infected individuals (Hosseini et al., 2020). Until now, studies have established a direct link between SARS-CoV-2 infection and its neurotropism. However, the vicious nature of cytokines and chemokines in response to activation of the innate immune response has not been sufficiently studied. Najjar et al. (2020) have discussed the detrimental effects of uncontrolled innate immunity on the function of the blood-brain barrier (BBB), which activates the CNS immune pathway and leads to the disruption of neural circuits. Further studies have discussed the potential of SARS-CoV-2 to target the respiratory centers in the brain, leading to massive inflammation and probable respiratory failure. Therefore, respiratory insufficiency is not merely associated with lung failure but could also result from the presence of interleukins, tumor necrosis factors, and other cytokines that impair the functioning of the medullary cardiorespiratory center in the brain (Li et al., 2020; Steardo et al., 2020).

Intriguingly, previous studies have shown the capacity of SARS-CoV-2, but not SARS-CoV, to replicate in U251 neuronal cells, highlighting the neuroinvasive potential of the virus (Chu et al., 2020). Thus, at this point, it became essential to test whether SARS-CoV-2 infects human neurons and productively replicates in the CNS. Indeed, experiments exposing SARS-CoV-2 to human brain organoids have revealed the presence of virus in neurons and other neuronal cell types (Ramani et al., 2020; Jacob et al., 2020; Pellegrini et al., 2020a; Pinar Mesci et al., 2020; Song et al., 2021). Remarkably, analyzing the brain autopsy samples of diseased COVID-19 patients, the Iwasaki lab has

Table 1. Neurological symptoms revealed by the clinical studies diagnosing COVID-19 patients

Neurological Indication	Clinical Symptoms	References
Headache	• dizziness • headache • impaired consciousness	(Asadi-Pooya and Simani, 2020)
Sensory impairment	• hyposmia • vision impairment • hypogeusia • dysgeusia	(Baig, 2020; Dell'Era et al., 2020; Lechien et al., 2020; Munhoz et al., 2020)
Guillain-Barré, skeletal, and neuromuscular syndrome	• ascending tetra paresis • paresthesia • areflexia • ataxia • bilateral abducens palsy • albuminocytologic dissociation • axonal demyelination • muscle weakness • higher creatinine kinase level • higher lactate dehydrogenase	(Alberti et al., 2020; De Sanctis et al., 2020; Gutierrez-Ortiz et al., 2020; Mao et al., 2020; Munhoz et al., 2020)
Encephalopathy	• meningoencephalitis • rhombencephalitis • altered mental status • hyperreflexia • posterior reversible encephalopathy • meningeal signs	(Munhoz et al., 2020; Wu et al., 2020; Yin et al., 2020)
Cerebrovascular disease	• cerebral hemorrhage • ischemic stroke • thrombocytopenia	(Beyrouti et al., 2020; Jin et al., 2020; Khan et al., 2014; Tang et al., 2016)
Altered mental status	• confusion • agitation • disorientation • delirium • apathy	(Baig, 2020; Varatharaj et al., 2020)
Seizures	• direct entry and infection into CNS • causes meningitis and seizure	(Narula et al., 2020)
BBB	• presence of SARS-CoV-2 in the brain microvascular endothelial cells •affects the integrity of the BBB • microvascular dysfunction	(Alquisiras-Burgos et al., 2020; Bohmwald et al., 2018)
Hemostatic abnormalities	• disseminated intravascular coagulation • severe inflammatory response	(Lu et al., 2020)
Immune response dysregulation	• causes cytokine storm • damage of CNS • formation of thrombosis • causes damage of BBB	(Alquisiras-Burgos et al., 2020; Baig, 2020; Mehta et al., 2020)

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Table 1. *Continued*

Neurological Indication	Clinical Symptoms	References
Brain anatomy irregularities	• vulnerable appearance of hippocampus • higher bilateral gray matter volume in the structures of central olfactory system • thrombotic microangiopathy • endothelial injury • alteration in the walls of the small arterioles, capillaries, and venules • disappearance of endothelial cells	(Hernandez-Fernandez et al., 2020; Lu et al., 2020; Ritchie et al., 2020)
Psychosis	• bizarre behavior • anxiety, panic disorder • suicidal ideation • agitation • suspiciousness • auditory hallucinations • alteration in personality, behavior, cognition	(Ferrando et al., 2020; Varatharaj et al., 2020)
Dementia	• post-infection memory impairment • delirium	(Ritchie et al., 2020; Wang, 2020)

identified the presence of SARS-CoV-2 in cortical neurons (Song et al., 2021). That work established the direct neuroinvasive capacity of SARS-CoV-2 in the human brain and substantiated the findings revealed in human brain organoids. Human brain organoids have allowed several groups to independently test the neurotropism of SARS-CoV-2 amid this pandemic. Here, we summarize the neurotoxic effects of SARS-CoV-2 revealed by human brain organoid research. We also highlight the obstacles incurred using human brain organoids and discuss how solving those limitations can help efficient modeling of COVID-19 and identify potential therapeutic agents against SARS-CoV-2.

What are human brain organoids and how do they help in understanding neurotropic viruses?

Brain organoids are innovative experimental model systems generated from either embryonic stem cells or induced pluripotent stem cells (iPSCs). With the combination of modern 3D cultures and directed differentiation methods, the Sasai and Vaccarino laboratories were the first to exploit the self-assembling properties of pluripotent stem cells and to generate 3D neural epithelial tissues (Eiraku et al., 2008; Kadoshima et al., 2013; Mariani et al., 2012; Mariani and Vaccarino, 2019; Nakano et al., 2012). When these neural epithelial tissues are cultured in spinner flasks or suspension cultures with defined media, they underwent self-organization to mimic *in vivo* tissue counterparts. Lancaster and colleagues named these objects 3D brain organoids as they mirror many aspects of neural epithelial tissues cytoarchitecturally, similar to the developing human brain (Renner et al., 2017; Lancaster et al., 2013). These organoids constitute vastly diverse cell types ranging from polarized radial glia, intermediate progenitors, to layer-specific cortical neurons (Quadrato et al., 2017; Birey et al., 2017; Gabriel et al., 2020; Pasca et al., 2015; Gabriel et al., 2016).

Further advances have generated region-specific brain organoids, such as midbrain, hypothalamus, cerebellum, brain organoids with light-sensitive cell types, vasculatures mimicking the BBB, and brain organoids with microglia (Cakir et al., 2019; Li et al., 2018; Monzel et al., 2017; Ormel et al., 2018; Pellegrini et al., 2020b; Qian et al., 2018; Silva et al., 2020). Several elegant review articles have summarized the historical development of brain organoid cultures and various revolutionary methods, which fine-tuned brain organoid cultures suitable for detailed questions that cannot be faithfully addressed *in vivo* models (Gabriel et al., 2020; Gopalakrishnan, 2019; Velasco et al., 2020). For instance, early brain organoids applicable to model neurodevelopmental disorders and mature organoids appropriate to model neurodegeneration-like effects (Gabriel et al., 2016; Grenier et al., 2020; Pavoni et al.,

Table 2. Neurotropic viruses characterized by the use of human brain organoids

Neurotropic Virus	Target	Major Finding	References
SARS-CoV-2	cortical neuron	• SARS-CoV-2 targets neurons in organoid • associates tau abnormalities and induces cell death	(Ramani et al., 2020)
SARS-CoV-2	neuron	• causes metabolic changes in the infected and neighboring neurons	(Song et al., 2021)
SARS-CoV-2	neuron, astrocyte, choroid plexus	• causes productive infection in choroid plexus organoids with cellular function deficiency	(Jacob et al., 2020)
SARS-CoV-2	choroid plexus	• damages brain choroid plexus and the blood-CSF barrier	(Pellegrini et al., 2020a)
Zika virus (ZIKV)	neural progenitor cells	• ZIKV isolates infect brain organoids triggering premature neural progenitor cell differentiation • teratogenic effects of ZIKV can be modeled in organoid • resembles microcephaly in organoids by decreasing neuronal cell layer volume • induces RNAi-mediated antiviral immunity • alters the DNA methylome of neural genes	(Gabriel et al., 2017) (Janssens et al., 2018; Qian et al., 2016; Watanabe et al., 2017; Xu et al., 2019)
West Nile virus (WNV)	neural stem cells	• WNV shows a higher replication kinetics in NSCS • aggressive cytopathic effects are observed in NSCs	(Desole et al., 2019)
Herpes simplex virus 1 (HSV-1)	peripheral nerve ganglia and CNS	• causes acute HSV-1 infection in brain organoid • showed features of latency • potential causative agent of Alzheimer disease (AD) • AD can be mimicked in organoid with HSV-1 induction	(Cairns et al., 2020; D'Aiuto et al., 2019)
Japanese encephalitis virus (JEV)	neural precursor cells and glial cells	• JEV tends to infect astrocytes and radial glial cells • innate antiviral immune response can be attained during cortical organoid development	(Khan et al., 2014; Tang et al., 2016; Zhang et al., 2018)

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Table 2. *Continued*

Neurotropic Virus	Target	Major Finding	References
Varicella zoster virus (VZV)	ganglionic neurons	• after infection, VZV becomes latent in ganglionic neurons • VZV does not induce apoptosis in the neurons	(Pugazhenthi et al., 2011)
Human immunodeficiency virus 1 (HIV-1)	microglia and other glial cells	• HIV-1 infects microglia of brain organoid • major hallmarks of HIV neuropathology were demonstrated by the organoid model	(Dos Reis et al., 2020)
Human cytomegalovirus (HCMV)	neural progenitor cells	• HCMV infection induces regional necrosis • presence of cyst and large vacuoles in organoids • altered neurological development has been observed	(Brown et al., 2019)

2018). Elaborating the in-depth detail is out of the scope of this article and, thus, we focus on how human brain organoids have helped us to underpin the mechanisms of neurotropic viral infections and, in particular, SARS-CoV-2, the current strain of interest.

Most probably, the brain organoids have earned their significant momentum while modeling the disease mechanisms of Zika virus (ZIKV) during the ZIKV epidemic. Microcephaly is a rare neurodevelopmental genetic disorder caused by mutations in centrosomal and cilia genes. However, there was a sudden rise in microcephaly in infants during the ZIKV epidemic associated with their infected mothers. While ZIKV-induced microcephaly mechanisms remained a mystery, brain organoids have immensely helped to identify their target cell types, consequences, disease mechanisms, and potential therapeutic agents (Gabriel et al., 2017; Qian et al., 2017; Watanabe et al., 2017). Likewise, studies have just started to appreciate brain organoids in understanding the mechanisms of human cytomegalovirus and herpes simplex virus (HSV), neurotropic viruses to which the CNS is vulnerable. In summary, all of these works have set up a stage on which human brain organoids are reliable model systems to study the infection mechanisms, target cell types, and neurotoxic effects of SARS-CoV-2. Human brain organoids are particularly instrumental for modeling COVID-19 because rodents have significant limitations to faithfully recapitulate human COVID-19 symptoms as they require overexpression of human ACE2 to facilitate viral entry and to exhibit COVID-19 phenotypes (Hoffmann et al., 2020; Winkler et al., 2020; Yang et al., 2007; Bao et al., 2020). Also, obtaining clinical tissues of human brain is generally difficult for obvious reasons, and this becomes even more complicated from patients with contagious diseases due to safety concerns (Hanley et al., 2020). Table 2 summarizes neurotropic viruses studied in human brain organoids.

State-of-the-art modeling of COVID-19 in human brain organoids

To date, only a small number of works have modeled the neurotropism and neurotoxic effects of SARS-CoV-2 in human brain organoids (Jacob et al., 2020; Pellegrini et al., 2020a; Pinar Mesci et al., 2020; Ramani et al., 2020; Yang et al., 2020; Zhang et al., 2020; Eric Song et al., 2020; Jacob et al., 2020). Ramani et al. exposed iPSC-derived brain organoids to SARS-CoV-2. During the early onset of the pandemic in March 2020, the authors could not acquire commercial anti-SARS-CoV-2 antibodies to detect the neurotropic effect of the virus in brain organoids. Instead, they generated convalescent plasma from recovered COVID-19 patients. The convalescent plasma that specifically recognized the spike protein of SARS-CoV-2 could label the virus in their organoids. Their findings revealed that the virus could directly target a moderate number of cortical neurons expressing pan-neuronal and cortical markers of TUJ-1 and tau (Ramani et al., 2020). The authors have tested two age groups of organoids falling 40 days apart and identified that the later age groups were much more susceptible to the viral entry. Notably, the tropism of SARS-CoV-2 in their experiments was largely limited to post-mitotic neurons, a property of the virus markedly

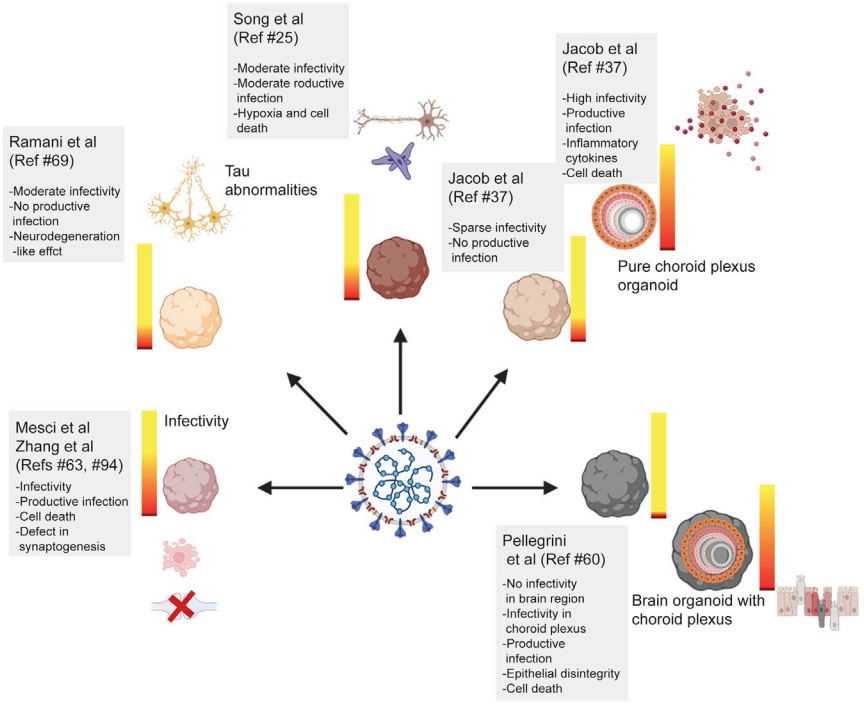

Figure 1. Schematic summarizes the effect of sARS-CoV-2 in brain and choroid plexus organoids

Gray boxes list the various cellular effects caused by SARS-CoV-2. The bars adjacent to organoid images show infectivity represented from yellow (low) to red (high).

differing from the ZIKV, which prefers proliferating neural progenitor cells (Gabriel et al., 2017).

Independent studies by Pinar Mesci, Song, Zhang, and Yang and colleagus (Pinar Mesci et al., 2020; Yang et al., 2020; Zhang et al., 2020) have also observed similar neurotropism for SARS-CoV-2 in their brain organoids or iPSC-derived neurons, i.e., the virus-targeting progenitors, neurons, and glial cells; however the findings of Pellegrini et al. (2020a) state that SARS-CoV-2 cannot target neuronal cell types (Figure 1). Furthermore, except for Ramani et al. (2020), some of these works showed a slight increase in viral production, suggesting that brain organoids could support the productive infection of SARS-CoV-2 (Jacob et al., 2020; Pinar Mesci et al., 2020; Song et al., 2021). In particular, Song et al. identified the ability of SARS-CoV-2 to target the brain organoids and detected a majority of the virus along MAP2-positive neurons, indicating the affinity of the virus for neuronal cells. Indeed, these MAP2-positive neurons exhibited the presence of ACE2, a known primary viral entry factor. Consequently, brain organoids pre-treated with anti-ACE2 antibodies reduced the viral entry, suggesting an influential role for ACE2 in neurons. Although ACE2, a known entry factor for SARS-CoV-2, is expressed in low levels in neurons, the finding showing that the viral entry and its replication occurs has raised a question that there may be additional neuron-specific viral entry factors besides ACE2 and TMPRSS2. Recently, Cantuti-Castelvetri et al. (2020) elegantly explained the role of neuropilin-1 (NRP1) in mediating efficient binding of the SARS-CoV-2 to ACE2 receptors and thereby viral entry. Thus, it is worth testing the expression and the role of NRP1 in brain organoids and various cell types derived from brain organoids.

Besides all of these, Jacob et al. (2020) systematically tested the neurotropism of SARS-CoV-2 in various region-specific brain organoids of cortical, hippocampal, hypothalamic, and midbrain regions (Figure 1). Agreeing with the findings of Ramani et al. (2020), Jacob et al. also did not identify a significant increase in viral production between 24 and 72 hours post-infection. Taken together, these studies, which employed neural organoids to some extent, indicate the viral tropism to human neurons, which is also convincingly substantiated by Song et al. (2021) detecting SARS-CoV-2 in the cortical neurons of COVID-19 patients. These findings do raise the basic question of how the virus could enter the neurons in the brain. One potential possibility is that SARS-CoV-2 has a tropism for nasal epithelial cells and can enter the CNS from the nasal cavity through the olfactory bulb (Sungnak et al., 2020; Montalvan et al., 2020).

On the other hand, the CNS is protected against inflammatory agents and pathogens by the BBB and the blood-CSF barrier. Thus, damage in these protective barriers may elicit a passive entry route for SARS-CoV-2. Intriguingly, analyzing hippocampal organoids, Jacob et al. (2020) identified transthyretin-expressing choroid plexus-like cells

that were densely positive for SARS-CoV-2. As the choroid plexus anatomically develops adjacent to the hippocampus, the authors speculated that choroid plexus cells are vulnerable to infection by SARS-CoV-2, disrupting the epithelial integrity of choroid plexus, thereby allowing viral entry into the neural epithelia. Inspired by this idea, Jacob et al. (2020) developed a robust and straightforward method for generating pure choroid plexus organoids. On exposing them to SARS-CoV-2, the authors have identified that the virus infects, productively replicates, and induces an inflammatory response and cell death. The work of Pellegrini et al. (2020a) also supported the strong tropism of the virus toward choroid plexus epithelia and determined that SARS-CoV-2 can damage the integrity of choroid plexus epithelial upon its entry. Thus, both of these works substantiate an exciting possibility that the choroid plexus epithelium is susceptible to infection, and that the virus can enter the CNS as a consequence of barrier damage.

Cellular consequences of the entry of SARS-CoV-2 revealed by brain organoids

Given the complex neurological defects observed in COVID-19 patients, ranging from headaches, dementia-like syndromes, to encephalitis, SARS-CoV-2-induced effects are rather multifactorial cellular defects. It is difficult to comprehend that 2D or 3D *in vitro* experimental models of neuronal cultures can mirror these effects. However, brain organoids have surprisingly revealed some useful insights. All of the works that have thus far modeled COVID-19 in brain organoids unambiguously showed that the neurons that harbored virus undergo cell death (Jacob et al., 2020; Pinar Mesci et al., 2020; Jacob et al., 2020; Ramani et al., 2020). Cell death phenomena are an end product, and the underlying cellular reasons are complex. Pinar Mesci et al. show in their experiment that SARS-CoV-2 entry is associated with impaired synaptogenesis, as assessed by a reduction in the proportion of VGlut1-positive neurons in the virus-positive cells, suggesting a potential long-term implication of SARS-CoV-2-induced synaptopathy-like effects.

On the other hand, Song and colleagues have suggested that virus entry could trigger cellular stress and neuronal cell death (Song et al., 2021). Strikingly, their work has convincingly demonstrated that the bystander cells in the vicinity of the virus-positive cells undergo cellular stress and death. The authors reasoned this by showing the appearance of hypoxia-induced factor I along with TUNEL-positive cells.

Ramani et al. (2020) tested whether SARS-CoV-2 could elicit neuronal stress, eventually inducing neuronal death. In particular, the authors noticed that SARS-CoV-2-positive neurons express tau, a cortical marker implicated in Alzheimer disease, and tauopathy-related neurodegeneration. Intriguingly, the authors identified that, although very few neurons were positive for the virus, most if not all have exhibited aberrant tau distribution. Tau is exclusively present in the axons, which surprisingly relocated to the cell soma of the virus-positive neurons. Such a tau missorting has been frequently observed in tauopathy-related disorders. Extending their analysis, they found that the virus entry is also associated with aberrant tau phosphorylation. In particular, they observed that the virus-positive neurons were specifically associated with tau phosphorylation at the Thr-231 site. Tau Thr-231 phosphorylation is one of the hallmarks of aberrant phosphorylation events inducing neuronal stress and tauopathy-like effects. Intriguingly, it has been documented that HSV-1 targets human neurons and causes aberrant tau phosphorylation and aggregation, typical of neurodegeneration (Alvarez et al., 2012; Wozniak et al., 2009). As discussed above, the observed neurotoxic effects cannot be merely caused by the virus directly but are also due to bystander effects, such as inflammatory response induced by infiltrating immune cells. This is due to the fact that SARS-CoV-2 infection has shown great potential in inducing a cytokine storm, which not only affects the lungs, but also other organs throughout the body.

On performing bulk RNA sequencing, Jacob et al. (2020) identified that, besides several factors, SARS-CoV-2-infected choroid plexus organoids express an increased level of inflammatory cytokines, tumor necrosis factor alpha, and several interleukins, indicating the triggering of an immune response. The authors also observed the downregulation of AQP1, AQP4, and SLC22A8, components that are critical to maintain the tight junctions of choroid plexus epithelia. In summary, although organoids are reductionist model systems, they have offered crucial insights into the disease mechanisms of COVID-19, which cannot easily be addressed in biopsy or living human brain samples.

Limitations of brain organoids in modeling COVID-19

It is essential to note that, until now, brain organoids used to model COVID-19 have been reductionist models containing mostly cell types for chosen differentiation conditions. These organoids, mainly at the proliferative state, are an almost perfect model system to study the effect of neurotropic viruses that target neural stem cells. COVID-19 clinical symptoms are rather degenerative-like effects occurring in adult brains harboring mature cell types. Even though the current-state-of-the-art brain organoids have shown that SARS-CoV-2 has a moderate tropism to cortical neurons, the use of brain organoids in COVID-19 modeling remains the tip of the iceberg. Thus, generating meaningful neurological COVID-19 modeling requires engineered brain organoids expressing mature cell types of astrocytes,

oligodendrocytes, myelinated neurons, and other neuronal cell types expressing ACE2, TMPRSS2, and NRP1.

Particular importance should be given to astrocytes and microglia, which may play significant roles in neuroinflammation in response to SARS-CoV-2 infection. It has become clear that cortical neurons will not be functional or viable without glial cell support. Astrocytes are glial cells that play critical roles in CNS homeostasis, synaptogenesis, and, most importantly, mediating immune response by interacting with microglia. Microglia, on the other hand, are the resident immune cells in the brain and function as first responders to brain infection. It is known that, upon viral infection, microglial cells are quickly activated to transmit pro-inflammatory molecules, reactive oxygen species, and activate astrocytes. Microglia are highly heterogeneous, and their full complement is unexpected in currently existing brain organoids since microglial cells do not originate from the neuroectoderm. However, efforts have been made to tailor brain organoids to generate microglia. For example, omitting SMAD inhibitors, Ormel et al. (2018) have shown the presence of reactive microglia in brain organoids, where Iba-1-positive cells overlap with PSD-95, a protein associated with neurons involved in synaptogenesis. Therefore, it would be interesting to test the levels of microglial cells in brain organoids in response to SARS-COV-2 infection. This would help in understanding the immediate effect of immune cells on the neighboring neurons *in situ*. Likewise, Ramani et al. (2020) also observed Iba-positive microglial cells and S100β-positive astrocytes in their differentiation protocols. Future experiments generating brain organoids with enriched levels of these cell types will help us dissect if the observed neuronal death is due to bystander effects of pro-inflammatory signals caused by these glial cells upon infection.

Another significant limiting factor in brain organoids is the lack of vasculature exhibiting characteristics of BBB. This is because one cannot exclude virus spillage into the CNS via a disrupted BBB. This route indeed favors access of cytokines and infected T cells and monocytes. A readily available example is HIV, another RNA virus possessing neurotropism, which has been a tremendous public health concern. HIV-1-infected T cells and monocytes cross the BBB, eventually targeting microglia and CNS perivascular macrophages (Bertrand et al., 2019). It remains unknown whether SARS-CoV-2 follows a similar strategy to target the CNS neurons. Thus, brain organoids with vasculature mimicking the microenvironment of BBB can help to address this question. Vasculature in brain organoids is unexpected, as endothelial cells do not originate from the neuroectoderm. Nonetheless, pessimism is not necessary. Recent advances in 3D organoid cultures have generated hybrid organoids of neuroepithelial tissues and vasculatures. Co-culturing of preformed vasculature and neurospheres has successfully generated these hybrid organoids. The In-Hyun Park lab (Cakir et al., 2019) took an innovative approach to generate functional vasculatures in the human brain organoids. By overexpressing ETS variant 2, a transcription factor that reprograms cells into endothelial cells, their protocol has generated brain organoids with functional vasculatures (Pham et al., 2018).

In summary, even though the current state-of-the-art brain organoid-based COVID-19 modeling is at the primitive stage, these efforts have been the impetus to advancement the field. It is plausible that brain organoids will reveal unprecedented details when the above-mentioned limitations have been satisfactorily addressed.

Future perspectives

The current SARS-CoV-2 pandemic has taken center stage in biomedical science. While the focus to date has been mostly on the respiratory system, the fact that about 30%–40% of COVID-19 patients develop detrimental CNS defects has surprised the world. These numbers have made us speculate as to what primarily causes patients to succumb to the disease? It is noteworthy that the SARS-CoV outbreak in 2003 stopped suddenly. Thus, studies of human brains from SARS-CoV-infected patients with CNS symptoms are lacking. Also, the currently available human brain organoids did not exist 15 years ago. Thus, our knowledge of the neurotoxicity of coronaviruses has been significantly limited.

Thanks to the recent research into human organoids, which has already given us critical insights into the neurotropism of SARS-CoV-2. Immediate experiments utilizing the mature state of brain organoids and complex brain organoids harboring vasculature, choroid plexus, and astrocytes ensure the comprehensive dissection of the neuropathology of SARS-CoV-2. Hopefully, these advances will enable brain organoids to reliably replicate the viral progenies. This will allow us to conduct drug-screening studies to identify therapeutic compounds that can stop viral entry, replication, and mitigate the CNS symptoms, which cause deterioration of the quality of human life. Identifying anti-SARS-CoV-2 agents is vital, especially given the current uncertainty with regard to identifying an effective vaccine.

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DECLARATION OF INTERESTS

The authors declare no competing interests.

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