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Modeling SARS-CoV-2 infection in individuals with opioid use disorder with brain organoids

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Abstract

The COVID-19 pandemic has aggravated a preexisting epidemic: the opioid crisis. Much literature has shown that the circumstances imposed by COVID-19, such as social distancing regulations, medical and financial instability, and increased mental health issues, have been detrimental to those with opioid use disorder (OUD). In addition, unexpected neurological sequelae in COVID-19 patients suggest that COVID-19 compromises neuroimmunity, induces hypoxia, and causes respiratory depression, provoking similar effects as those caused by opioid exposure. Combined conditions of COVID-19 and OUD could lead to exacerbated complications. With limited human in vivo options to study these complications, we suggest that iPSC-derived brain organoid models may serve as a useful platform to investigate the physiological connection between COVID-19 and OUD. This mini-review highlights the advances of brain organoids in other neuropsychiatric and infectious diseases and suggests their potential utility for investigating OUD and COVID-19, respectively.

Keywords

COVID-19, SARS-CoV-2, opioid use disorder, neuropsychiatric disease, brain organoid

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Introduction

The worldwide COVID-19 pandemic, caused by the SARS-CoV-2 virus, arrived in the midst of another epidemic: the opioid crisis. Opioid use disorder (OUD), a substance use and addictive disorder defined in part by the *Diagnostic and Statistical Manual of Mental Disorders, 5th Edition* (5th edition; DSM-5) as the desire to take opioids despite social and professional consequences, currently affects 40.5 million people worldwide, but the opioid crisis was actually beginning to decline as more people were gaining access to effective treatment options.[1–3]

COVID-19 may reverse this trend, though, as people with OUD often experience significant challenges in healthcare that could be aggravated by the pandemic.[1] For example, quarantine and social distancing measures could disrupt both vital social support groups and medication for addiction treatment, which is generally administered in person.[4] Those with OUD could also be more likely to contract COVID-19 due to cognitive impairment, lower awareness of risk, and diminished efforts regarding personal protection in patients, further disrupting treatment.[5,6] Such premature

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treatment cessation dramatically increases overdose rates. In addition, the rise in fear, anxiety, and depression caused by COVID-19, induced by medical and financial instabilities, can be especially harmful to people with pre-existing mental health conditions and can cause relapses, worsening of these conditions, and increased substance usage.[7,8] Indeed, since the rise of COVID-19, the United States has seen an increase of 32% for non-prescribed fentanyl and an 11% increase in drug overdose deaths in the first 4 months of 2020 as compared with those of last year.[9]

Although there is much discussion on the circumstantial ramifications of COIVD-19, SARS-CoV-2 infection itself might prove to be directly challenging for those with OUD, who generally show worse overall health and increased susceptibility to infectious disease complications.[10]

COVID-19 is primarily known to induce sequelae such as respiratory distress syndromes and multiple organ dysfunction syndromes, but clinical observations indicated that patients with more severe cases also developed neurological and neuropsychiatric manifestations targeting the central nervous system (CNS), including dizziness, impaired consciousness, encephalitis, ataxia, acute cerebrovascular disease, and taste/smell impairment.[11,12] Furthermore, long-term COVID-19-related psychiatric symptoms are still unknown; the significantly elevated prevalence of mental health issues in survivors of SARS-CoV, including posttraumatic stress disorder (PTSD), depression, and anxiety, took years to develop.[13,14] These could further affect those with OUD who, in addition to addiction, typically suffer from comorbid psychopathologies such as depression, anxiety, and dizziness.[15,16]

Some of the neurological symptoms in the patients with COVID-19, such as encephalitis, may be caused by neuroimmunologic responses that stimulate inflammatory damage in the CNS.[13] Similarly, opioids bind to various opioid receptors in the CNS to modulate the neuroimmune system, stimulating both high levels of pro-inflammatory and neurotoxic cytokines (IL-6, IL-1β, TNFα) and those that are anti-inflammatory and neuroprotective (IL-10, TGF-β, and BDNF).[17,18] In addition, risks of opioid-related respiratory depression are likely amplified by COVID-19.[19] SARS-CoV-2 infiltration in subcortical respiratory control centers could lead to observed hypoxia and respiratory distress.[20] Opioids also target the brainstem to cause dysregulation of respiratory and cardiac functions, leading to damaging hypoxic consequences in the brain and other tissues.[21] Moreover, the primary drugs prescribed for OUD treatment, methadone and buprenorphine, are partial opioid receptor agonists that could cause significant respiratory distress, the effects of which could be exacerbated by SARS-CoV-2 infection, unanticipated drug-drug interactions with novel COVID-19 therapeutics, and recently imposed self-administration of these highly potent treatments.[19,22–24]

The potential physiological connection between OUD and COVID-19 has been implicated in peripheral immune function as well. Opioid usage can have differential immune system responses, causing both immunosuppressive and immunostimulatory effects on cytokine secretion and chemokine receptor activation.[10,25,26] SARS-CoV-2 demonstrates similar adverse effects on peripheral immune responses. It causes a lack in Interferon Type I/III (IFN-1/3) response while simultaneously inducing high levels of chemokines, suggesting COVID-19 severity is tied to a cytokine storm.[27,28] These peripheral responses may also elicit neurological symptoms through precipitating neuroinflammatory responses or disrupting neurotransmission.[13]

Currently, our knowledge of how those with OUD may respond to COVID-19 is limited due to lack of research. However, based on the observations described above (Figure 1), we fear that sustaining COVID-19 and OUD could lead to especially serious complications and more complex interventions because of the effects that both have in compromising neuroimmunity and inducing hypoxia and respiratory distress. Our fears are not unfounded, as both COVID-19 and OUD patients with comorbidities have poorer prognoses and more severe cases.[27,29–31]

Animal models, important to study pathology, transmission, and host responses, have been used to investigate both SARS-CoV-2 infection[32,33] and opioid addiction (Table 2). However, physiological differences among species provoked speculation that established animal models lack of good correlates for SARS-CoV-2. Indeed, animal models have historically led to a particular failure in neuropsychiatric drug development.[34–37] An animal model to study both OUD and COVID-19, though significant, would still bear both sets of limitations. Because human in vivo investigation is difficult due to ethical considerations, the safety of handling patients with SARS-CoV-2, and the limitation in identifying patients who have had both OUD and COVID-19,[35] human tissue-based models will provide critical validation steps to fill in our knowledge gap. A large portion of understanding both the individual and combined effects of OUD and COVID-19 on the brain will thus fall on these in vitro models that best mimic human physiology.[38]

Organoids, self-assembled three-dimensional (3D) aggregates generated from human induced pluripotent stem cells (iPSCs), most effectively recapitulate the cytoarchitecture and functional features of native tissues.[39] Researchers have recognized organoids' superior ability to mimic viral pathophysiology and investigate pharmacodynamics, and they have sprinted to develop in vitro organoid models to evaluate the response and drug candidates in COVID-19 cases.[40]

In this mini-review, we will briefly describe brain organoids and their advantages. We will then highlight the advances of brain organoids in neuropsychiatric and infectious diseases to understand their role in investigating OUD and COVID-19, respectively. Last, we will discuss their potential to study COVID-19 infection in those with OUD.

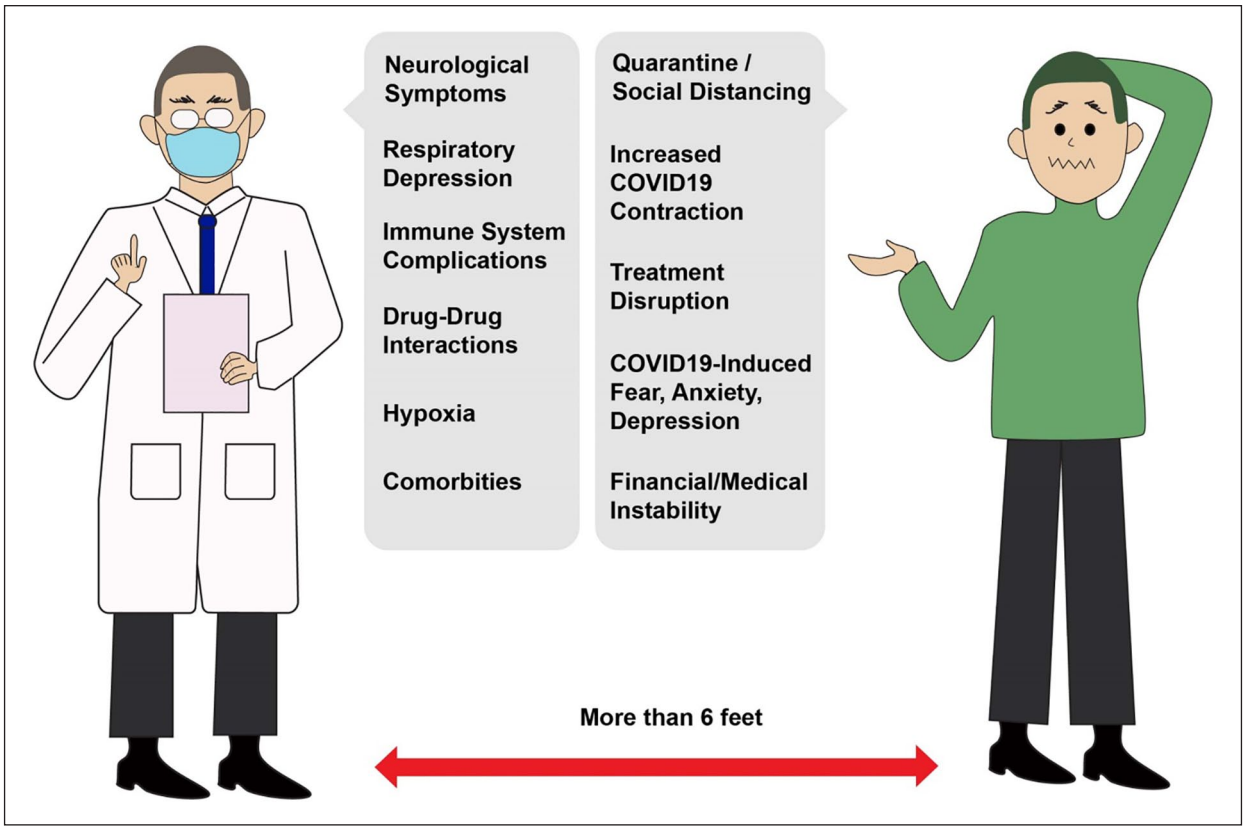

Figure 1. Illustration summarizing the circumstantial complications (right, patient) and SARS-CoV-2 infection complications (left, physician) that the COVID-19 pandemic can potentially have on those with OUD.

What are brain organoids?

Understanding the human brain, with both its vast number of specialized cell types and complex connectivity, has been historically challenging.[41,42] Knowledge of the biological bases of neuropsychiatric disorders is unsatisfactory, too, and human health continues to suffer.[43] This reality is primarily due to our limited access to the healthy human brain; most of our knowledge is built on human pathological or post-mortem specimens, animal studies, and in vitro 2D culture models. These models, although invaluable, are unable to capture the full scope of the living human brain, and they are limited by inherent species differences, concerns over tissue availability and manipulation, and lack of cell diversity and structural organization.[34,36,41] Advancing neuroscience will require better human tissue-based models that can recapitulate the developmental and functional dynamics of human brain.

Recent advances in cellular reprogramming and stem cell culture techniques have enabled the in vitro generation of 3D brain organoids from an individual's unique genetic background.[44,45]

Brain organoids are self-assembled 3D cellular aggregates that are generated from embryonic stem cells (ESCs) or iPSCs to mimic the brain. These brain organoids, which contain a 3D-organized heterogeneous cell population, can partially recapitulate some of the brain's structure, developmental stages, and functionality, such as synapse formation and intercellular signal transmission.[46–48] In addition, brain organoids can model specific regions of the brain; a variety of protocols demonstrate the generation of brain organoids to model the development of cortex,[49–52] hypothalamus,[51] midbrain,[53–55] and cerebellum.[56] To generate brain organoids, iPSCs can be derived via the reprogramming of somatic cells. iPSCs can self-organize to form embryoid bodies (EBs). These EBs could then be cultured in the presence of neural induction molecules and region-specific patterning factors to give rise to brain organoids modeling specific brain regions (Figure 2). Each protocol details a distinct cocktail of patterning molecules (e.g. SMAD inhibitor and WNT3A) and generates brain organoids with cellular heterogeneity and spatial architecture. For instance, cerebral organoids consist of upper-layer and deep-layer neurons, radial glia cells, and neural progenitors that display a rosette-like structure mimicking the subventricular-like zone.[50]

In addition, brain organoids can be easily accessed for live imaging of temporal and spatial changes, drug responses, electrophysiological activities, and high throughput transcriptomic and proteomic profiling.[41,52,57–61]

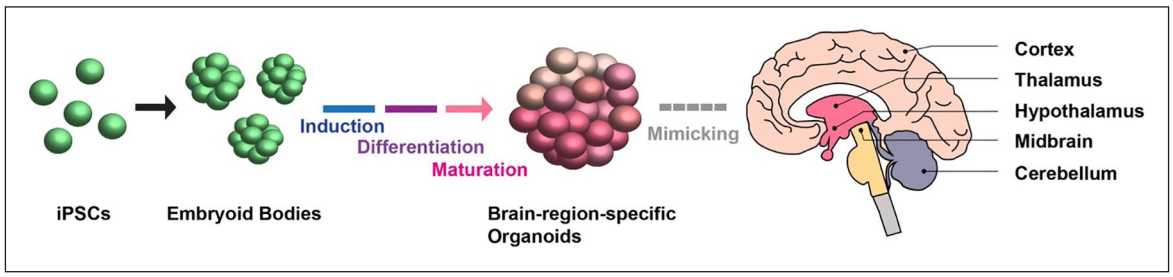

Figure 2. Schematic representation of general brain organoid generation. Human iPSCs self-assemble into embryoid bodies, and then sequentially undergo induction, differentiation, and maturation to form brain organoids. These organoids can mimic specific brain regions and model corresponding functional studies. Neurobiological mechanisms of various brain disorders can be investigated using patient-derived or genetically mutated iPSCs, as well as with the appropriate brain region.

The mechanics of certain mutations or disorders can then be understood by comparing control organoids to those bearing specific genetic modifications or originating from patients. With these strategies, in addition to the advantages provided by a 3D platform, brain organoids hold promise as relevant and powerful tools to model human brain development and disease.

Brain organoid models for OUD and neuropsychiatric diseases

OUD is part of the wide range of neuropsychiatric disorders in which neurodevelopmental features and neurological functions have been comprised.[62–64] OUD and other substance use disorders are usually compounded with additional neuropsychiatric disorders, such as depression, anxiety, schizophrenia, bipolar disorder, attention-deficit hyperactivity disorder (ADHD), psychotic illness, borderline personality disorder, and antisocial personality disorder.[65–68] Researchers have posed several reasons for such a high prevalence of comorbidity between OUD and other neuropsychiatric disorders: (1) they target similar brain regions (nucleus accumbens, ventral tegmental area, prefrontal cortex, hippocampus, amygdala) and neural circuitry (implicating the reward system, decision making, impulse control, stress response, and emotions)[66–68]; (2) they share common molecular mechanisms affected by various genetic, epigenetic, and environmental factors such as genetic mutations, stress, adversity, trauma, and drug exposure and/or access[62,65–67,69–71]; (3) there are numerous clinical similarities and overlapping symptoms with each other, and one disease may unmask or exacerbate the symptoms of the other.[67] However, the precise mechanisms for many neuropsychiatric diseases, including OUD, are unclear, and further investigation is crucial for a greater understanding of the nature of these diseases and their complex relationship.

Although OUD has yet to be investigated using brain organoids, many studies have been conducted on other neuropsychiatric diseases. These studies successfully investigated similar pathways and regions that would be relevant to OUD, suggesting that brain organoids can provide a viable framework for studying human brain disorders and explore potential therapeutics. To show the power of brain organoids for OUD, we will first highlight several significant advances that brain organoids have made in a few kinds of neuropsychiatric disorders: neurodevelopmental, psychotic, mood, and neurodegenerative.

Neurodevelopmental disorders

The first neuropsychiatric disease to be studied using brain organoids was autism spectrum disorder (ASD), a developmental disorder impairing social interaction, communication, and behavior.[72,73] ASD patient-derived telencephalic organoids recapitulated previously observed impaired neurodevelopment, increased inhibitory interneuron differentiation, synaptic overgrowth, and cellular overproduction.[72,74–76] These organoids additionally demonstrated that inhibiting upregulated FOXG1, important for telencephalic development, rescued normal morphology. Later studies further revealed the specific temporal sequence of ASD-specific molecular and phenotypic abnormalities, additional genes and regulatory elements underlying ASD onset, enriched PI3K–AKT–mTOR signaling, and enlarged organoid sizes.[73,77,78] Others found that chromodomain helicase DNA-binding protein 8 (CHD8) haploinsufficiency and RAB39b mutations, previously hypothesized to be top ASD candidate genes, was sufficient for provoking ASD-like phenotypes in organoids.[77,79–81]

Other neurodevelopmental disorders were soon studied using brain organoids, too. Rett syndrome, which causes intellectual disability and ASD-like behavior, is characterized by mutations in the X-linked gene MeCP2 that governs microRNA regulation.[82] Patient- and MeCP2 knockdown-derived cerebral organoids yielded upregulated miR-199/miR-214 expression and transcriptional dysregulation altering human neuronal development.[83,84] In addition, genetic pathways and pharmacological treatments for chromosome 16p13.11 microduplication, which is associated with several neurodevelopmental disorders, were able to be assessed in cerebral organoids.[85,86] Last,

the known imbalances in excitatory and inhibitory neurotransmission found in Down Syndrome were shown to be recovered by shRNA-mediated knockdown of OLIG2 in patient-derived forebrain organoids.[87,88]

Psychotic disorders

Schizophrenia is characterized by many symptoms, including delusions, hallucinations, amotivation, anhedonia, and cognitive deficits, that are thought to arise from disrupted neurodevelopment.[89,90] Patient-derived organoids confirmed the hypothesized impaired telencephalic neuronal development, attributing it to reduced FGFR1 expression, and also demonstrated aberrant immune response, mitochondrial function, increased TNFα and decreased Wnt signaling, and excitatory/inhibitory neurotransmission.[91–95]

In addition, mutations in DISC1(disrupted-in-schizophrenia 1) have been established as a genetic risk factor for various psychiatric disorders including schizophrenia, but the mechanism of how DISC1 disrupts brain function is not well known.[96,97] Cerebral organoids suggested that disturbing the interaction between DISC1 and Ndel1/Nde1 impairs cell-cycle progression during mitosis.[98] Organoids also revealed that the smaller rosette structures, elevated WNT signaling, disorganized layer specificity, and dysregulated cell fate could be rescued by correcting the DISC1 mutation or using a WNT antagonist.[99,100]

Mood disorder

Previous investigation into bipolar disorder (BD), a psychiatric disorder that is characterized by recurring episodes of depression and mania, have implicated certain genes and impairments in BD patients.[101–105] Forebrain organoids corroborated these findings and also exposed dysregulated nervous system development, differentiation, immune signaling, and electrical stimulation.

Neurodegenerative disorders

Alzheimer's disease (AD), associated with severe age-related memory impairments, is a neurodegenerative disorder characterized by amyloid plaques, neurofibrillary tangles, and tau pathology (taupathy).[106] Raja et al. first demonstrated that these AD pathologies could be replicated in patient-derived organoids, and treatment of these organoids with β- and γ-secretase inhibitors, known AD therapeutics, significantly reduced amyloid and taupathy.[107] CRISPR/Cas9-altered organoids showed that organoids with apolipoprotein E4 (APOE4), a known risk factor in AD, recapitulated multiple AD phenotypes.[108,109] More investigations revealed further mechanisms.[110–117]

Many other neurodegenerative disorders have been investigated with brain organoids, too. Midbrain organoids successfully recapitulated the abnormal differentiation and increased cell death of dopaminergic neurons reminiscent of Parkinson's disease (PD).[118,119] A later study also identified that the LRRK2 G2019S gene mutation associated with PD causes α-synuclein accumulation, mitochondrial dysfunction, increased neurotoxicity, and impaired dopamine signaling.[120] In addition, cerebral organoids confirmed the role of p25/Cdk5 in human taupathy, characteristic of frontotemporal dementia.[121] Last, it has been suggested that Huntington's disease, an inherited neurodegenerative disease caused by an expansion of CAG repeats in the huntingtin gene, is caused by earlier abnormal development.[122,123] Huntingtin's disease patient-derived cerebral organoids modeled this irregular neural development, demonstrating failure of neuroectodermal acquisition, abnormal neural rosette formation, disrupted cell organization, impaired cortical fate differentiation, and terminal neuronal maturation.[124] Molecular and pharmacological approaches targeting the huntingtin mutation restored striatal normalcy, suggesting that an early intervention may revert neurodegeneration later in life.

The above studies, summarized in Table 1, demonstrate the viability of using brain organoids to interrogate the disease biology for a wide range of neuropsychiatric disorders. Although OUD itself has yet to be investigated with brain organoids, the approaches described in these studies provide a roadmap for future interrogation of OUD.

Molecular mechanisms of opioid exposure on the brain were revealed by transcriptome profiling and its downstream annotation analysis (Table 2). RNA sequencing (RNA-seq), along with downstream Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analyses, help find affected biological processes and pathways. These experiments have revealed common signaling pathways and biological processes shared between OUD and these related neuropsychiatric disorders. Brain organoids would provide a more nuanced understanding of these processes particular to OUD, including specific gene targets, morphological abnormalities, and therapeutic possibilities.

An example of a mutual biological process would be the dysregulated immune functioning and inflammation found in several neuropsychiatric disorder organoid studies, which is partially due to impaired IFNγ, NF-κB, and other signaling pathways.[86,93,102,109] Karagiannis et al. similarly found that acute and chronic opioid exposure impairs the antiviral gene program through genes related to IFN cell signaling,[174] while others have shown that deviant pro- and anti-inflammatory cytokine generation is due to aberrant toll-like receptor (TLR) and NF-κB signaling.[153,155,188] It has further been observed that heroin-dependent patients (HDPs) exhibit lessened cytokine secretion due to the downregulation of TNFα signaling pathways.[151,189,190]

In addition, brain organoid studies have revealed imbalanced excitatory/inhibitory neurotransmission,[72,73,88,93] which could further elucidate how chronic opioid exposure increases glutamatergic signaling.[153,154] Furthermore, HDPs shared commonly upregulated genes with those who have PTSD, AD, PD, and HD, including those related

Table 1. Studies investigating neuropsychiatric and infectious diseases using 3D brain organoids.

Disease (Neuropsychiatric and Infectious)	Region-specific brain organoid used	Key findings	Reference(s)
Autism spectrum disorder	Telencephalic Cerebral Forebrain	Increased mature inhibitory neurons Abnormal cellular differentiation Neurodevelopmental temporal dysregulation Enhanced synaptic development Alterations rescued by FOXG1, CHD8-KO, RAB39b-KO Enriched PI3K-AKT-mTOR signaling Enlarged organoids	Mariani et al.,[72] Schafer et al.,[73] Amiri et al.,[77] Zhang et al.,[78] Wang et al.[79]
Rett syndrome	Cerebral	Altered neurogenesis and neural differentiation Increased ventricular area and decreased radial thickness in organoids Rounder cells, reduced processes Upregulated miR-199 and miR-214	Mellios et al.,[83] Xiang et al.[84]
16p13.11 Microduplication	Cerebral	Reduced NPC proliferation, rescued by targeting NFκB p65 pathway Smaller organoids Altered cellular organization	Johnstone et al.[86]
Down syndrome	Forebrain	Increased OLIG2+ ventral forebrain neural progenitors Increased inhibitory interneurons Altered neurodevelopment	Xu et al.[88]
Schizophrenia	Cerebral	Disrupted cellular organization and cell migration Impaired neurodevelopment Several disrupted signaling pathways, including TNFα and Wnt Transfection of FGFR1 rescued abnormalities Dysregulated genes related to ECM	Stachowiak te al.,[91] Kathuria et al.,[93] Benson et al.,[94] Sawada et al.[95]
DISC1 mutation	Forebrain Cerebral Cortical	Mutation disrupted DISC1-Ndel1/Nde1 interaction Delayed cell-cycle progression Smaller rosettes, disorganized cell layers Alterations rescued by WNT antagonist	Ye et al.,[98] Srikanth et al.,[99] Qian et al.[100]
Bipolar disorder	Cerebral (patterned after dorsal forebrain)	Impaired cell adhesion, neurodevelopment, synaptic biology, and immune signaling Abnormal response to electrical stimulation	Kathuria et al.[102]
Alzheimer's disease	Cerebral Neuroectodermal Forebrain	Amyloid aggregation, hyperphosphorylated tau protein, endosome abnormalities Altered synaptic function, lipid metabolism, immune and inflammatory responses, neural differentiation, electrical activity, ECM composition, mitochondrial unfolding and clearance Increased apoptosis and cell death APOE4 recapitulated AD phenotypes β- and γ-secretase inhibitors, DAPT, heparin, and heparinase reduced pathology	Lambert et al.,[108] Lin et al.,[109] Pavoni et al.,[110] Gonzalez et al.,[111] Meyer et al.,[112] Arber et al., [113] Ghatak et al.,[114] Perez et al.,[115] Yan et al.,[116] Zhao et al.,[117]

(Continued)

Table 1. (Continued)

Disease (Neuropsychiatric and Infectious)	Region-specific brain organoid used	Key findings	Reference(s)
Parkinson's disease	Midbrain	Altered development of midbrain dopaminergic neurons Increased apoptosis, α-synuclein inclusions, FOXA2 expression G2019S mutation in LRRK2 recapitulated PD phenotypes	Smits et al.,[118] Kwak et al.,[119] Kim et al.[120]
Frontotemporal dementia	Cerebral	Reduced levels of phosphorylated tau and increased expression of synaptophysin upon blocking p25 generation	Seo et al.[121]
Huntington's disease	Cerebral Cortical	Disrupted cytoarchitecture, neural development Alterations rescued by downregulation of mutant HTT	Conforti et al.[124]
Zika virus	Cortical Cerebral Forebrain	Disrupted neurogenesis, neurodevelopment Increased cell death, decreased organoid size and cortical folding Several potential viral entry receptors and therapeutics were found and tested to prevent infection	Qian et al.,[51] Cugola et al.,[125] Garcez et al.,[126] Dang et al.,[127] Watanabe et al.,[128] Ayala-Nunez et al.,[129] Gabriel et al., [130] Li et al.,[131] Sacramento et al.,[132] Setoh et al.,[133] Wells et al.,[134] Xu et al.,[135,136] Yoon et al.,[137] Zhang et al.,[138] Zhou et al.,[139] Janssens et al.,[140] Nowakowski et al.,[141] Li et al.[142]
La crosse virus	Cerebral	Increased apoptosis Viability rescued by recombinant Type I IFN	Winkler et al.[143]
Japanese Encephalitis virus	Telencephalon	Infected astrocytes and outer radial glial cells Increased cell death, decreased cell proliferation	Zhang et al.[144]
Human Cytomegalovirus	Forebrain Cerebral	Abnormal neurodevelopment and neural layering Decreased organoid size Dysregulated calcium	Sison et al.,[145] Guoqiang et al.[146] Brown et al.[147]
Herpes simplex virus-1	Cerebral	Impaired neural differentiation, cortical layering, brain regionalization Abnormal microglial proliferation and activation Increased inflammatory factors TNFα, IL-4, IL-6, IL-10	Qiao et al.[148]
Sporadic Creutzfeldt-Jakob disease	Cerebral	Increased metabolism, LDH release, and cytokine release	Groveman et al.[149]
Toxoplasma Gondii	Cerebral	Replicated complex asexual life cycle, including tissue cysts Infected neuronal cells, astrocytes, oligodendrocytes Revealed differential gene expression, including enriched IFN-I response	Seo et al.[150]

Neuropsychiatric diseases are in bold, and infectious diseases are in italics.

Table 2. Affected biological processes and pathways by opioids and corresponding literature support.

Category	GO/KEGG term	Species	Drug	Effects
Neural function	Alzheimer's disease	Human	Heroin[151]	White matter degeneration in the brain[152]
	Amphetamine addiction	Mouse	Morphine[153]	Nociceptive sensitization, hyperalgesia[154]
	Calcium signaling pathway	Mouse	Morphine[155]	Analgesia[156,157]
	Cell projection morphogenesis	Mouse	Morphine[155]	Structural plasticity[158]
	Cell morphogenesis involved in neuron differentiation	Mouse	Heroin[155]	Neural plasticity[159]
	Ensheathment of neurons	Mouse	Morphine[155]	Synaptic plasticity, fast impulse conduction[160–162]
	Glutamatergic synapse	Mouse	Morphine[153]	Nociceptive sensitization, hyperalgesia[154]
	Huntington's disease	Mouse Human	Morphine, Heroin[151,163]	Synaptic plasticity[163]
	Long-term potentiation	Mouse	Morphine[153,163]	Tolerance, dependence[164]
	Nicotine addiction	Mouse	Morphine[153]	Nociceptive sensitization, hyperalgesia[154]
	Parkinson's disease	Human	Heroin[151]	Increased apoptosis[120]
	Potassium ion transport	Mouse	Heroin[155]	Proanalgesic effect[156]
	Regulation of circadian rhythm	Mouse	Morphine[153]	Modulated pain sensation[153,165]
	Regulation of excitatory postsynaptic membrane potential	Mouse	Heroin[155]	Tolerance, hyperalgesia[156]
	Regulation of nervous system development	Mouse	Heroin[155]	Neural plasticity, tolerance, dependence[166]
	Regulation of neuron death	Mouse	Heroin[155]	Anti-inflammatory effect, analgesia[166–168]
	Regulation of programmed cell death	Mouse	Morphine[155]	Production of pro-inflammatory cytokines, tolerance, hyperalgesia[154,169]
	Regulation of synapse organization	Mouse	Heroin[155]	Analgesia, autophagy[156,170]
	Synaptic transmission, GABAergic	Mouse	Morphine[155]	Pro-inflammatory responses, tolerance, affective control[169,171,172]
	Synaptic transmission, glutamatergic	Mouse	Morphine[155]	Analgesia, conditioned place preference memory acquisition[156,173]
	Voltage-gated ion channel activity	Mouse	Morphine[153]	Nociceptive sensitization, hyperalgesia[154]
Immune response	Defense response to virus	Human	Heroin[151]	Adverse effect in immune system[151, 174]
	Humoral immune response	Mouse	Morphine[155]	Anti-inflammatory effect, analgesia[175,176]
	Immune response	Mouse	Oxycodone[177]	Pro-inflammatory responses[177]
	Inflammatory response	Mouse	Oxycodone[177]	Anti-inflammatory effect, analgesia[175,176]
	Innate immune response	Human Mouse	Heroin, Morphine[155,151]	Pro-inflammatory responses, tolerance[156,169,178]
	MAPK signaling pathway	Mouse	Morphine[155]	Pro-inflammatory responses, analgesia, hyperalgesia, analgesic tolerance[179–181]
	Negative regulation of NF-κB TF activity	Mouse	Morphine[155]	Analgesia, NF-κB activation[169]
	Response to xenobiotic stimulus	Mouse	Morphine[155]	Tolerance[175,182]
	TNF signaling pathway	Human	Heroin[151]	Impaired hematopoiesis and circulation[151]
	Toll-like receptor signaling pathway	Mouse	Morphine[155,153]	Allodynia, hyperalgesia, NF-κB activation[180,183–187]

to oxidative phosphorylation and metabolic pathways (ATP5D, ATP5H, HSD17B10/NDUFB11, NDUFA8, NDUFB7).[151] Opioid exposure additionally caused dysregulated programmed cell death, synapse organization, cell morphogenesis, brain volume, and neuron differentiation,[152,169,191] similar to several of the above brain organoid studies.[72,73,83,86,91,93,98–100,124]

Although these animal models and human postmortem opioid studies are revealing, these experiments were designed to study specific functions and are limited in investigating the effects of opioids. Brain organoids provide unique access to human tissue that could be manipulated and assessed in order to expound on these effects of opioid exposure and inform on more precise mechanisms. Synthesizing the above opioid and brain organoid studies would be helpful to conduct further research on OUD.

To begin this further investigation, we tested the response to opioid exposure of niche cells in midbrain organoids, which were generated using previously described protocols.[53] Our preliminary analyses suggested that brain organoids are a powerful model for recapitulating both human brain development and drug response. First, chronic opioid usage arrested the development of neural progenitors, delaying the formation and maturation of the neuroblasts and neurons. This is comparable to the previous mouse studies that showed the inhibition of embryonic neurogenesis in the developing cortex due to morphine exposure, and a rebound increase in radial glial cell and neural progenitor proliferation and differentiation resulted from drug withdrawl.[192–194] Second, opioid exposure downregulated inflammatory responses, which is in line with its role in neuroinflammatory responses in both peripheral and central nervous systems.[195] Third, opioids led to alterations in neurotransmission and neural activity, which is consistent with their regulation of neurotransmitters through opioid receptors and downstream cAMP signaling pathways.[196] Interestingly, our results suggested that opioid exposure effects organoids in a cell-type specific manner as well. For instance, compared to untreated midbrain organoids, genes associated with synapse assembly and ion transport were more frequently altered in opioid-treated oculomotor and trochlear nucleus (OMTN) neurons, while genes associated with neuron development were less frequently altered in opioid-treated radial glia cells. These findings suggest that brain organoids are viable models for OUD and could further identify OUD-specific neuronal pathways and biological processes, as well as screen for potential drug candidates.

This section has reviewed the literature demonstrating that brain organoids are viable models to investigate some key aspects of OUD and neuropsychiatric diseases. In addition, our group's current project using midbrain organoids to study OUD has been encouraging. However, there is still much more to learn using these models, such as genetic variants prone to addiction, precise neuropathological mechanisms, and drug-drug interactions, in order to better understand both OUD and these complex diseases.

Brain organoid models for COVID-19 and infectious diseases

Although COVID-19 has presented neurological symptoms, and SARS-CoV-2 has been detected in patients' cerebrospinal fluid (CSF), the precise mechanisms of neurological infection and its complications are largely unclear.[197] The low expression of angiotensin-converting enzyme 2 (ACE2), a protein crucial for SARS-CoV-2 viral entry, in the CNS has evoked considerations that there are other mechanisms of action for neurological invasion.[11,198,199] Suggested mechanisms include retrograde neuronal transport, exosomal cellular transport, hematogenous routes, immune injury, and hypoxia damage.[11,12,198,200–203] Further studies, however, are required to determine both neuropathogenesis and neurotropism.

Brain organoids have successfully been used to study several infectious diseases and their effects on the human brain, giving hope for brain organoids to do the same for COVID-19.

The prime example for this is Zika virus (ZIKV), a virus that is associated with microcephaly and congenital brain malformations; during the global health emergency starting in 2015, brain organoids were essential to help determine its neuropathogenesis.[204–206] ZIKV successfully infected brain organoids, which additionally demonstrated that it causes microcephalic phenotypes such as cell death and reduced organoid growth.[51,125,126,142] Brain organoids were then used to suggest viral entry receptors that potentially cause microcephaly.[127,128,141] Many successive studies have since used transcriptomics and morphology to investigate ZIKV molecular pathways, neurotropism, and potential therapeutics.[129–139] A recent study even found that ZIKV alters DNA methylation and could potentially cause delayed-onset neuropsychiatric complications.[140]

Other infectious viruses have been studied with brain organoids, too. La Crosse Virus (LACV) is an arthropod-borne orthobunyavirus that causes many symptoms such as pediatric arboviral encephalitis, learning and memory deficits, and seizures.[207,208] Cerebral organoids inoculated with LACV showed increased apoptosis, where committed neurons underwent apoptosis at a higher rate than neural progenitors.[143] The susceptibility to infection was found to be due to poor IFN-1 response in less mature neurons, and exogenous administration of IFN-1 induction rescued this cell viability. Japanese encephalitis (JE), a disease caused by the Japanese encephalitis virus (JEV) with no effective cure for infected patients, causes bleeding and inflammatory infiltration in multiple brain regions, as well as longer-term neurological sequelae.[209,210] Telencephalon organoids found that JEV preferentially infects astrocytes and outer radial glial cells, stunts cell proliferation, and induces cell death.[144] Human Cytomegalovirus (HCMV) infection modulates intracellular calcium signaling and can result in infants born with a variety of symptoms, including hepatosplenomegaly, microcephaly, and developmental disabilities.[211,212]

Organoids revealed the mechanisms altering calcium signaling and also demonstrated disrupted cortical neural layering, terminal differentiation, cell viability, and organoid growth.[145–147] These studies also determined potential cellular receptors relevant to HCMV infection, and demonstrated that certain viral inhibitors and neutralizing antibodies could restore normal structural features. Neonatal herpes simplex virus type 1 (HSV-1), a member of the herpesviridae family, can cause various neurodevelopmental disabilities and necrotizing encephalitis that last well into adulthood.[213,214] HSV-1-infected cerebral organoids confirmed that the virus impairs neuronal differentiation and cortical layering, as well as revealed the abnormal increase in microglial proliferation and specific inflammatory factors.[148]

Brain organoids have even been exploited to investigate non-virus pathogens. Sporadic Creutzfeldt-Jakob Disease (sCJD), a transmissible neurodegenerative disease targeting several brain regions and caused by infectious prions, was shown with cerebral organoids to increase metabolism, LDH release, and cytokine release.[149,215,216] *Toxoplasmosa gondii* (TG), an intracellular protozoan parasite with no vaccine or effective treatment, can cause several CNS diseases and neurodevelopmental defects such as mental retardation, seizures, and microcephalus.[150,217,218] The unusual asexual life cycle of TG, which alternates between tissue cyst-forming bradyzoites and inflammation-inducing tachyzoites, requires a heterogeneous cell population for replication that has had underwhelming results in 2D cultures.[150, 218–220] Cerebral organoids were able to stimulate the complex TG asexual life cycle, including parasitophorous vacuoles that form cysts, and revealed that TG preferentially infects neuronal cells, astrocytes, and oligodendrocytes and primarily evokes an immune response from IFN-1.[150]

These studies have established brain organoids as a viable experimental infectious model, as summarized in Table 1. Because of this potential to also study SARS-CoV-2 CNS viral entry, which requires ACE2 expression, researchers are sprinting to develop in vitro organoid models to evaluate tropism and drug candidates in COVID-19 cases of tissues at risk (i.e., those that express ACE2).[198,199,221,222] Since it has been shown that both brain tissue[11,223] and brain organoids[58,61,224] express ACE2, brain organoids have recently been identified to study the neurotropism and neuropathogenesis of COVID-19, too. Very little is known with regards to SARS-CoV-2 neurotropism, but early results from several brain organoid studies have since provided some information.[225–231]

Although all studies have demonstrated that brain organoids were susceptible to SARS-CoV-2 infection, reports vary as to which specific cell types were indeed infected. One study reported that only cortical neurons were infected as opposed to neural progenitors, suggesting that SARS-CoV-2 prefers more mature neuronal cell types (Figure 3(a)).[225] Other studies, however, observed that SARS-CoV-2 infected other cell types, too, including neural progenitors, radial glia, dopaminergic neurons, and astrocytes.[226–230] Upon observing that choroid plexus (CP) epithelial cells had a much higher tropism than neurons in cortical organoids, two studies developed CP organoids and demonstrated a heightened SARS-CoV-2 viral spike protein expression in CP epithelial cells (Figure 3(b)).[227,231] All together, these studies suggest that there is some degree of neurotropism in COVID-19.

These studies also investigated the connection between cell-specific tropism and ACE2 expression. RNA sequencing demonstrated that only small amounts of ACE2 were found in neurons, neural progenitors, and astrocytes, with much higher expression found in CP epithelial cells and especially in the CP organoids.[225–228,230,231] However, immunofluorescence staining showed a more widespread expression of ACE2, indicating that ACE2 may be expressed on the cell surface and that mRNA levels were not necessarily the best representation of ACE2 amount (Figure 3(d)).[226] Although some studies found an overlap between SARS-CoV-2 positive cells and ACE2 expression,[227,231] others found no correlation between the two.[226,228] In addition, some studies found that there was a time-dependent increase in SARS-CoV-2-positive cells, indicating that the virus was able to replicate in brain organoids (Figure 3(a)),[226,227,229,231] whereas other studies found that the virus could not productively replicate.[225,227] Regions with robust infection were also found to have syncytia, caused by cell-cell fusion through the interaction between SARS-CoV-2 spike protein and ACE2 expressed on adjacent cells.[227,229] Song et al. also found that SARS-CoV-2 infection was inhibited by ACE2 antibodies (Figure 3(d)).[226] These results indicate that although ACE2 might be a critical cell entry receptor for CNS infection, it is likely that there are also other routes of infection that need to be further investigated. For example, cell-cell fusion was reported with SARS-CoV-2 infection of several cell types and was a major mechanism by which the virus spreads to adjacent cells.[232–234]

Brain organoids also revealed significant neurotoxicity caused by SARS-CoV-2 infection. TUNEL and cleaved caspase 3 immunolabeling demonstrated that SARS-CoV-2 infection causes significant cell death as compared with control organoids (Figure 3(c)).[225–227,229,230] Some observed this cell death even in uninfected cells adjacent to infected cells, suggesting that infected cells may induce adjacent cells to die through an extrinsic mechanism.[226,227] Several different mechanisms could have contributed to the observed neurotoxicity. SARS-CoV-2 impaired synaptogenesis and induced a locally hypoxic environment in neuronal regions, which aids in lowering the threshold for tissue damage.[226,230] In addition, cell-cell junctions of the epithelial cell layer of the choroid plexus, an integral part of the blood-CNS barrier that prevents the entry of pathogens, immune cells, and cytokines into the CSF and brain, were visibly damaged.[231,235,236] Such barrier breakdown

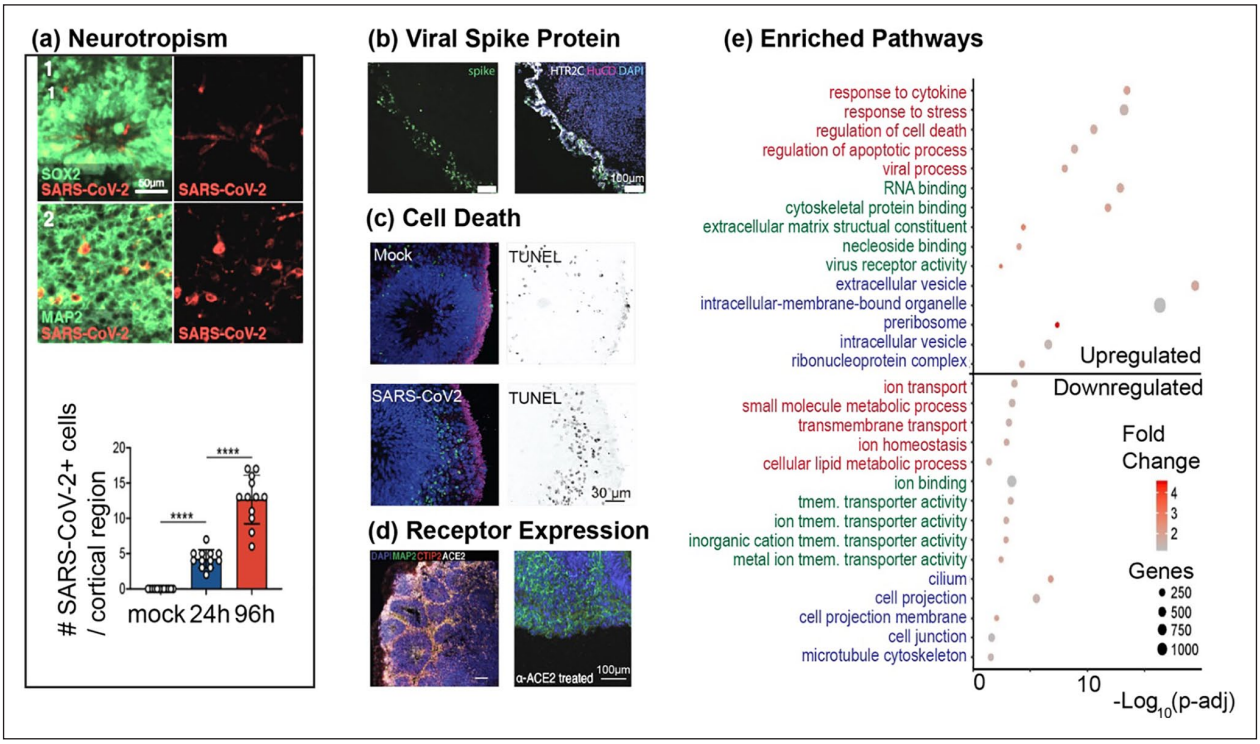

Figure 3. (a) Images of brain organoids revealing SARS-CoV-2 infection in both MAP2 mature neurons and SOX2 neural stem cells (top). Quantification shows increase in SARS-CoV-2 positive cells in the organoids, suggesting productive replication (bottom). Adapted with permission.[226] (b) Images of telencephalic organoids immunostained for HTR2C choroid plexus epithelial cells and HuCD neurons. Staining for SARS-CoV-2 viral spike protein expression reveals a much higher infection in choroid plexus cells than in other cortical cells and neurons. Adapted with permission.[231] Copyright 2020, MRC Laboratory of Molecular Biology. (c) Images of TUNEL-positive cells in control (top) versus SARS-CoV-2-exposed (bottom) organoids reveal SARS-CoV-2 positive cells experienced significantly higher cell death. Adapted with permission.[225] Copyright 2020, The Authors. (d) Immunofluorescence staining of ACE2 in brain organoids showed expression of ACE2 in MAP2-positive neurons (left). Immunofluroescence staining of organoids pre-incubated with anti-ACE2 antibodies (right) and infected with SARS-CoV-2 showed ACE2 antibody inhibited SARS-CoV-2 infection. Adapted with permission.[226] (e) Selected enriched GO terms for SARS-CoV-2 versus control organoids, including biological process (red), molecular function (green), and cellular components (blue). Adapted with permission.[227] Copyright 2020, Elsevier.

could allow abnormal entry of immune cells and cytokines, leading to harmful neuroinflammation.

Furthermore, brain organoids were found to display tau missorting, where tau is mislocalized in the somatodendritic region rather than on the axons of mature neurons, and tau hyperphosphorylation.[225,237] Taupathy is reminiscent of AD and other neurodegenerative diseases, suggesting that perhaps COVID-19 can cause chronic or long-term damage in the CNS.

Single-cell RNA sequencing, along with gene ontology analyses, further revealed the neuropathogenesis of SARS-CoV-2, identifying dysregulated cell division, inflammatory responses, cellular function, and metabolic processes (Figure 3(e)).[226,227] In addition, pathways in both infected and uninfected cells were enriched, but in different ways, and dysregulated gene expression varies widely among various tissue organoids infected with SARS-CoV-2. This suggests unique responses in different cell types and highlights the need for diverse cellular model systems when studying the disease.

A few different therapies to inhibit SARS-CoV-2 infection were tested, too. Sofosbuvir, an FDA-approved anti-hepatitis C treatment that suppresses viral families of single-stranded, positive-sense RNA viruses such as coronaviruses, successfully decreased the amount of neuronal cell death, viral accumulation, and synaptic damage.[230,238,239] In addition, SARS-CoV-2 infection was inhibited by both anti-ACE2 antibodies and CSF-containing antiviral antibodies.[226] Overall, brain organoids have provided a lot of information about SARS-CoV-2 neurotropism, and these models are established as a viable method to continue further and necessary investigation.

Summary and perspectives

As outlined in the introduction, there are a lot of potential complications between OUD and COVID-19. Both present neurological and psychiatric manifestations, cause respiratory depression, immune system dysfunction, and hypoxia, and are both aggravated by comorbidities.

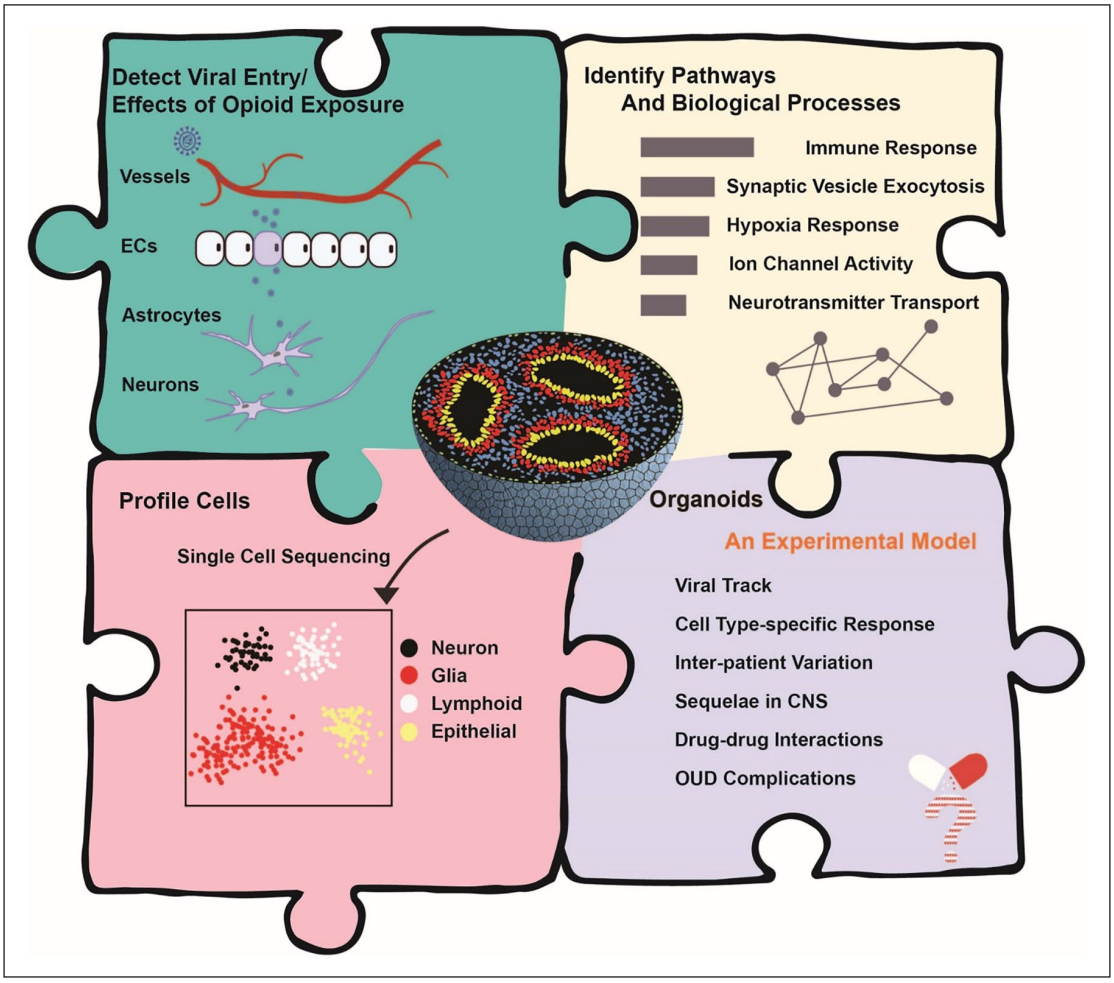

Figure 4. Brain organoids as an experimental virology and opioid abuse model of human CNS to detect viral entry, identify affected pathways, and profile neural cells.

Brain organoid studies investigating OUD and COVID-19, as well as related neuropsychiatric disorders and infectious diseases, have revealed further potential complications. Our preliminary study of OUD using midbrain organoids demonstrated that opioid exposure impairs neuronal differentiation and activity, as well as induces hypoxic and alters immune functioning in the CNS. It has been shown that SARS-CoV-2 infection, too, impairs neuronal viability and differentiation, while also stimulating a hypoxic and inflammatory CNS environment. In addition, the synaptic damage and tau pathologies associated with SARS-CoV-2 are reminiscent of similar findings in neurodevelopmental and neurodegenerative disorders, arousing concern that COVID-19 could cause long-term CNS damage.

It would thus be prudent to investigate COVID-19 complications in individuals with OUD through brain organoids, which could be accomplished using one of the following approaches: (1) generate organoids with OUD patient-derived iPSCs, and subsequently inoculate them with SARS-CoV-2; (2) generate organoids with COVID-19 patient-derived iPSCs, and subsequently expose them to opioids and recapitulate the effects of OUD; (3) generate organoids from control iPSCs and both inoculate them with SARS-CoV-2 and expose them to opioids. If the first, ideal option cannot be accomplished, the second and third options can also provide fundamental mechanistic insights.

Brain organoids can be used to investigate both the individual and combined effects of OUD and COVID-19 on the brain. Each model can be characterized and validated using similar tactics to the ones we exemplified (Figure 4). For instance, high-throughput proteomic and transcriptomic profiling could identify affected neuronal pathways and biological processes on a single cell level, revealing the particular difficulties COVID-19 contributes to those with OUD. Additional cell-type specific SARS-CoV-2 viral entry could also be tracked to develop novel COVID-19 drug candidates. Brain organoids hold potential to screen these candidates both individually and in conjunction with OUD therapeutics, such as methadone and buprenorphine.[240] Histological analysis could further demonstrate the effects of these diseases on morphology, architecture, electrophysiological function, and cellular

heterogeneity. We suggest that brain organoids would reveal how COVID-19 leads to worsened phenotypes and more complicated interventions in those with OUD.

Although brain organoids are extremely helpful, limitations do exist, such as incomplete cell maturation and heterogeneity, brain tissue architecture, transcriptional networks, and vasculature.[34,47,48,241] With the rapid evolution of brain organoid technology, we expect that broader aspects of COVID-19 complications can be investigated. For example, using improved brain organoid generation protocols that promote the yield of microglia, astrocytes, and endothelial cells with ACE2 expression, more accurate viral pathology and mechanisms of infection could be investigated.[225,241,242] In addition, since brain organoids more closely resemble the developing fetal brain than the mature adult brain, there may exist important differences in SARS-CoV-2 susceptibility between immature and mature cells.[227] Organoids with better maturity and cell and tissue type diversity are paramount to a better understanding of the interactions between COVID-19 and OUD, along with the development of more efficacious interventions.

Challenges in understanding the complications of COVID-19 is huge and urgent, especially to monitor long-term CNS consequences.[228] It will need interdisciplinary efforts from both clinical and basic studies. Hopefully, brain organoid and other established modeling can provide complementary information to improve our understanding and treatment of OUD in the wake of other viral infections and complications.

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