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COVID-19 lessons from the dish: Dissecting CNS manifestations through brain organoids

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Abstract

COVID-19 is increasingly understood as a systemic disease with pathogenic manifestations beyond the respiratory tract. Recent work by Ramani *et al* (2020) dissects the cellular and molecular mechanisms of SARS-CoV-2's neurotrophic properties, using viral exposure of human brain organoids. Their findings highlight neurons as primary target of cerebral SARS-CoV-2 infection and uncover its Tau-related neurotoxicity.

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See also: **A Ramani *et al*** (October 2020)

Our understanding of COVID-19 pathogenesis has been advancing rapidly, thanks to a global research effort of unprecedented scale and speed: from the mechanisms through which SARS-CoV-2 spike protein interacts with the ACE2 receptor and the host proteases TMPRSS2, cathepsin L and furin to infect host cells, to the dysregulation of the adaptive immune response of patients, including the cytokine storm that causes acute respiratory distress syndrome in severe cases and the emerging appreciation of the major involvement of the myeloid compartment (Schulte-Schrepping *et al*, 2020). Also the central nervous system (CNS) has been emerging as a major site of COVID-19 vulnerability, with a wide a range of manifestations that include dizziness, headache, impaired consciousness, acute cerebrovascular disease, ataxia, seizures, encephalitis and parkinsonism (Ellul *et al*, 2020). While this is not surprising considering that also SARS-CoV and MERS-CoV are neurotropic and exhibited neurovirulent properties, major gaps of knowledge remain about the respective traits of SARS-CoV-2 to understand, and thus hopefully prevent and treat, the neurological and psychiatric manifestations of COVID-19. This is mainly due to the difficulties in examining the relevant CNS cells from patients, and the challenges of recapitulating the dynamics of infection and its consequences in physiopathologically relevant experimental models.

The recent advent of brain organoids represents a versatile tool to overcome these obstacles (Kim *et al* 2020). These three-dimensional structures recapitulate the key developmental stages of human brain development; therefore, the application of brain organoids in the context of biomedical research has already proven its value to understand the pathogenesis of neuropsychiatric disorders, to unravel the unique regulatory mechanisms of human neurogenesis (López-Tobón *et al*, 2019), and to study the impact of infectious diseases on human brain, as in the paradigmatic case of Zika virus (Gabriel *et al*, 2017).

In their current work, Ramani *et al* (2020) exposed two developmental stages (Day 15 and Day 60) of human brain organoids to SARS-CoV-2 and followed the consequences over up to 6 days, assessing the number of infected cells by labeling with a virus-specific antibody, following an analogous design they previously applied to Zika virus (Gabriel *et al*, 2017). In contrast to what had been observed with the Zika virus, which mainly targeted neuronal progenitors, here SARS-CoV-2 showed a specific tropism for neurons and determined a higher number of infected cells in older (Day 60) organoids, where the percentage of mature neurons is higher than in the younger (Day 15) organoids. Importantly though, at least through 6 days exposure tested, the infection was not productive, either in 3D or in 2D neuron-only cultures, pointing to potential viral restriction mechanisms in neurons that limit the active replication of SARS-CoV-2. Finally, Ramani *et al* (2020) also found a striking impact of SARS-CoV-2 infection on brain organoid neurons, represented by the displacement of the physiological localization of the microtubule-associated protein Tau and increased cell death. The fact that in infected neurons Tau is mislocalized from the axons (the physiological localization in neurons of non-infected organoids) to the soma sheds new light on SARS-CoV-2 neurotoxicity and suggests the possibility of dysregulated pathways shared with the neurodegenerative disorders characterized by the hallmarks of tau pathology.

Within the emerging and understandably still sketchy knowledge context on SARS-

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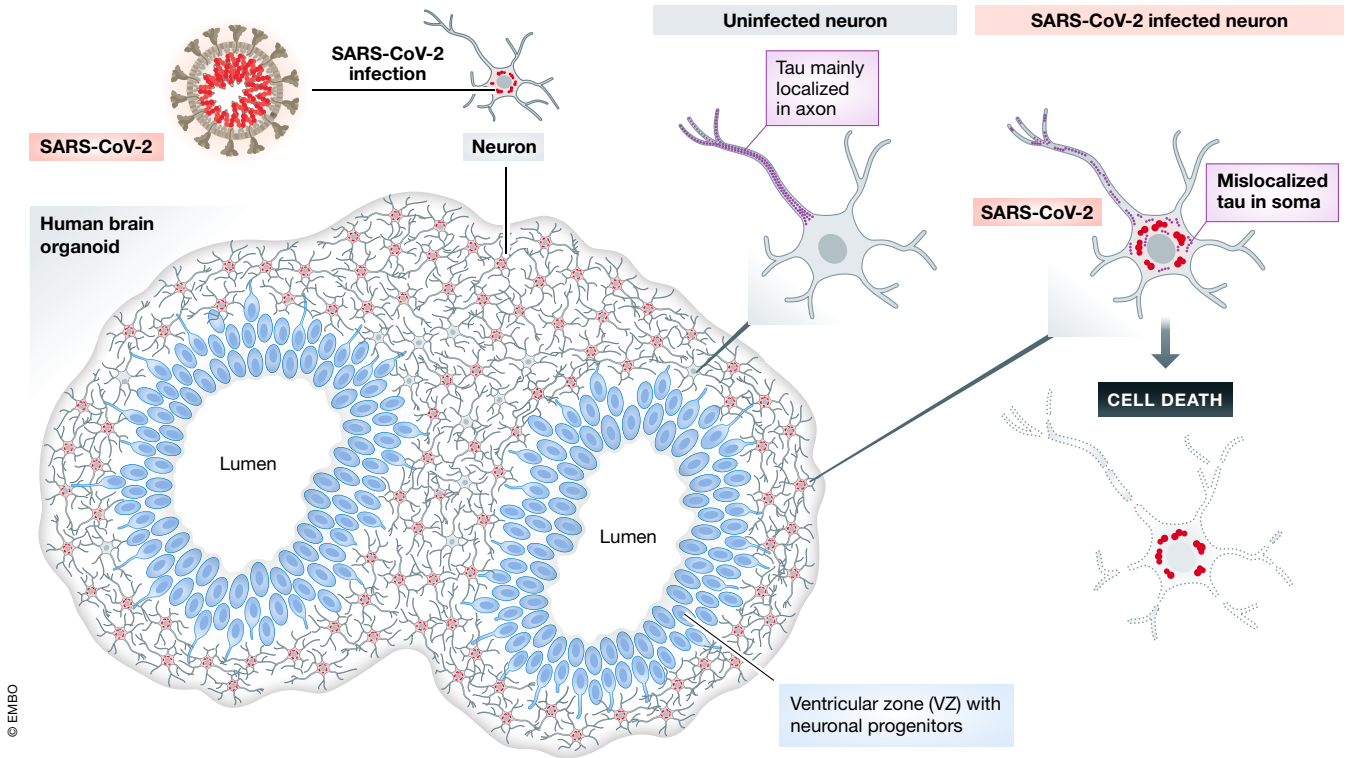

Figure 1. Schematic illustration of the neurotrophic properties of SARS-CoV-2, as demonstrated by viral exposure on human brain organoids.
Through this experimental system, the displacement of the physiological localization of the microtubule-associated protein Tau and increased cell death in infected neurons were identified as molecular mechanisms mediating SARS-CoV-2 neurotoxicity.

CoV-2 neuropathology, these findings highlight three main themes for an organoid-centered research agenda on COVID-19 and the brain, underscoring the crucial interplay between organoid and single-cell omic technologies that we and others recently proposed for patient-specific, interceptive medicine across the main health domains (Rajewsky *et al*, 2020).

The first is the topography of SARS-CoV-2 vulnerability in the brain. Notably, a related recent study that also exposed brain organoids to SARS-CoV-2 found choroid plexus cells, rather than neurons, as the main entry points of infection (Pellegrini *et al*, 2020), while in a yet different study neuronal progenitors in brain organoids were also shown to be targeted by the virus (preprint: Song *et al*, 2020). Besides potential differences due to protocol and detection specificities, such diversity may well point to the complexity of host-viral dynamics in the heterogeneity of brain tissue, warranting the use of multiple types of brain organoids and also at much later stages, so as to increase the representation of cell types and map, by single-cell profiling (including spatial transcriptomics), the impact of SARS-CoV-2 on human brain tissue. A panel of more complex, later stage brain organoid systems is thus poised to shed light on the fundamental question of neuro-COVID-19, namely the interplay between direct versus indirect virus-induced damage (e.g., inflammatory changes to the blood brain barrier). This also relates to the routes of brain infection, with olfactory neurons representing the most likely entry point for CNS retrograde spread, but with other routes that involve the vascular system and a hyperinflammatory state also needed to be investigated in greater detail.

Second, benchmarking with SARS-CoV-2 infected brain tissue will be key, again ideally in matched single-cell omic *ex vivo–in vitro* comparisons, since brain organoids currently recapitulate, at the very most, late prenatal/perinatal neuronal maturation milestones, while neuro-COVID19 concerns the adult brain and there is currently no evidence of an impact of SARS-CoV-2 on the developing human brain (given the scale of the pandemic Zika-like effects would have certainly been spotted by now). The need for ex vivo benchmarking is underscored by the neuropathological observations just released from the thus far largest patient's cohort, which found neuroinflammation of the brainstem as the most common finding (the study did not examine the cortex though) but claimed no evidence for CNS damage directly caused by SARS-CoV-2 (Matschke *et al*, 2020), consistently with the findings of (preprint: Song *et al.*, 2020) that observed the presence of the viral spike protein in the neurons of patients' post-mortem brains without an accompanying focal immune response (Fig 1).

Third, the observation that SARS-CoV-2 neuronal infections is not productive (or only minimally so; Ramani *et al*, 2020) warrants the use of genome-wide and/or targeted CRISPR screens to understand neuronal viral restriction and deconvolve the host factors permitting the first round of SARS-CoV-2 neuronal infection, as recently exemplified by the work of (Daniloski *et al*, 2020) on human alveolar epithelial cells.

Furthermore, given the emerging relevance of genetic variation for COVID-19 severity, applying such designs to iPSC-based brain organoids would allow to authentically validate neuropathogenic pathways in vulnerable patients' own genetic backgrounds. Finally, when also brain organoids become amenable to high throughput settings, the integration of genetic perturbations with drug screenings will be key to discover and/or repurpose compounds for preventing and treating CNS related COVID-19 symptoms, as exemplified by the finding of Sofosbuvir as a potential alleviator of neurological symptoms (preprint: Mesci *et al*, 2020).

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