The Wuhan wild-type strain and five variants of SARS-CoV-2 differently affect the viability of various brain cells (pericytes, astrocytes, endothelial cells, and microglia) and the integrity of the blood-brain barrier | 1

A significant number of individuals with COVID-19 or the post-acute phase of COVID-19 exhibit symptoms of central nervous system (CNS) disorders. The rise in the number of patients with neurological sequelae underscores the importance of identifying the mechanisms of the CNS infection and the CNS regions mostly affected by neuroCOVID. The authors from the United Kingdom conducted this study to investigate whether a wild-type form of SARS-CoV-2 (Wuhan strain) and five variants of concern/interest, the Alpha, Beta, Delta, Eta, and Omicron, differently affect cells of the CNS and modulate CNS infection.

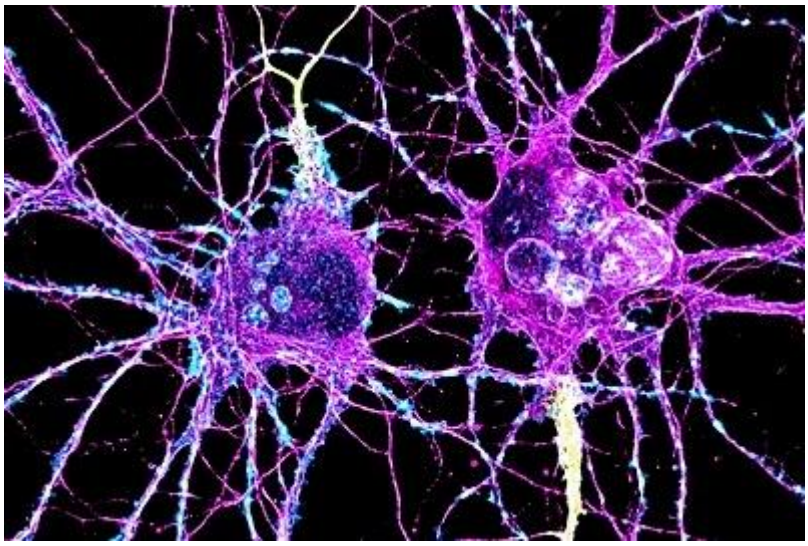
The neurological symptoms in COVID-19 patients range from mild symptoms such as headaches, confusion, insomnia, peripheral neuropathy, orthostatic intolerance, and syncope to more severe disorders such as stroke, extrapyramidal and movement disorders, encephalitis or encephalopathy, seizures, acute disseminated encephalomyelitis, Guillain-Barré syndrome, or hemorrhagic myelitis.
https://discovermednews.com/hemorrhagic-myelitis-after-the-sars-cov-2-infection/

The blood-brain barrier (BBB) and blood-cerebrospinal fluid (CSF) barrier are highly complex networks that protect the CNS parenchyma from harmful elements, including viruses. Certain viruses can overcome these obstacles by infecting vascular endothelial cells. This facilitates the direct passage of viruses through these barriers to the CNS. It should be noted that certain CNS structures, such as the choroid plexus and the circumventricular organs, including the hypothalamus, are not completely protected by the BBB and can serve as virus entry points.
https://discovermednews.com/the-involvement-of-hypothalamic-circuits-in-sars-cov-2-infection-of-the-central-nervous-system/ The principal components of the BBB are microvascular endothelial cells, but pericytes and astrocytes are the ultimate control of the barrier phenotype. Two host-cell factors are important for SARS-CoV-2 viral entry into many cell types: angiotensin-converting enzyme 2 (ACE2), which is bound by the SARS-CoV-2 spike (S) protein, and transmembrane protease, serine 2 (TMPRSS2), which cleaves the S-protein, allowing this binding to take place.

Neuroinvasion has been documented for almost all the β-CoVs, including SARS-CoV, MERS-CoV, and SARS-CoV-2, resulting in a similar spectrum of symptoms. The viruses enter the CNS *via* different pathways, including the olfactory pathway by which viruses reach the olfactory bulb via the olfactory nerves, the BBB, blood-CSF barrier, retrograde axonal transport, and the use of infected hematopoietic cells as “Trojan horses”. Viruses can use the olfactory and hematogenic pathways simultaneously. As clinical studies failed to detect significant levels of viral RNA in the CSF, CSF as a major transportation route for SARS-

CoV-2 has been questioned.

The olfactory bulb is considered the main gateway for viral entry into the brain. After intranasal inoculation, the viral antigens were detected in CNS regions that possess first- or second-order neural connections with the olfactory bulb, including the cerebral cortex, basal ganglia, midbrain, and hypothalamus.
https://discovermednews.com/retinal-inflammation-after-intranasal-infection-with-sars-cov-2/



About the Study and Results

In this *in vitro* study, the researchers investigated the impact of the wild-type Wuhan strain and the Alpha, Beta, Delta, Eta, and Omicron variants on the functional activities of primary human pericytes, fetal astrocytes, endothelial cells, and microglial cell lines and on a 3D model of the BBB.

ACE2 receptors were expressed only by astrocytes and microglia, and to a lesser extent by endothelial cells. Pericytes did not express ACE2. Since only the Omicron variant replicated in pericytes, which did not express ACE2 receptors, it indicates that the Omicron variant utilizes an alternative receptor to enter those cells.

The serine protease TMPRSS2 was expressed in all cell types, although pericytes exhibited a lower expression level. Astrocytes, endothelial cells, pericytes, and microglia expressed CD147 and neuropilin-1 (NRP-1), which interacts with the SARS-CoV-2 S protein as a co-

receptor for viral entry. According to the authors, the expression of ACE2, TMPRSS2, CD147, and NRP1 on astrocytes and microglia suggests a potential SARS-CoV-2 entry into those cells.

The results further revealed that the susceptibility and permissivity of brain cells to SARS-CoV-2 infection were dependent on the SARS-CoV-2 variant. The wild-type Wuhan strain and the Alpha, Beta, Delta, and Omicron variants caused a productive infection of brain cells. Infections caused by the Alpha, Beta, Delta, and Omicron variants were more extensive than those caused by the wild-type Wuhan strain.

The viability of pericytes, compared to other analyzed cells, was more affected by viruses. The Alpha and Beta variants were cytopathic only for pericytes. The Wuhan wild-type strain was cytopathic for pericytes and microglia. The Wuhan strain disrupted the endothelial barrier, inducing excessive and potentially lethal stress to those cells and decreasing microglial metabolic activity. The Omicron variant was cytopathic for pericytes and endothelial cells. The Delta and Eta variants decreased the metabolic activity of astrocytes.

All SARS-CoV-2 variants increased the mitochondrial activity in endothelial cells and pericytes, either temporarily (wild-type Wuhan strain) or continuously (Alpha, Beta, Delta, Eta, and Omicron variants). The Alpha and Beta variants caused a slight increase in astrocyte mitochondrial activity, and only the Alpha variant caused a slight increase in microglial mitochondrial activity. According to the authors, these findings suggest stress in those cells upon exposure to SARS-CoV-2.

Importantly, all SARS-CoV-2 variants, except the Beta, altered the extracellular concentration of glutamate, the main excitatory neurotransmitter in the mammalian CNS. The Omicron decreased, whereas other variants increased the extracellular concentration of glutamate (Delta continuously and the Wuhan wild-type, Alpha, Eta transiently).

In vitro BBB model

The Wuhan wild-type strain, and to a lesser extent, the Omicron variant, disrupted the integrity of the BBB. The other SARS-CoV-2 variants did not exhibit any effect. All SARS-CoV-2 variants, except the Eta variant, had a negative impact on one or more tight or adherens junction proteins, which play a crucial role in maintaining the integrity and selective permeability of the BBB. The most affected junctional protein was VE-cadherin which was significantly decreased by the wild-type Wuhan strain, and by the Alpha and Delta variants.

Conclusion

This study showed that the wild-type Wuhan strain and five variants, Alpha, Beta, Delta, Eta, and Omicron, which carry specific mutations that modulate their infectivity and transmissibility, affect the brain cells and the BBB differently.

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