A significant number of individuals with COVID-19 or the post-acute phase of COVID-19 have 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 CNS regions implicated in the pathogenesis of neuroCOVID. In this theoretical article, the authors from the United Arab Emirates and Australia discussed the possible pathways and mechanisms of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) neuroinvasion, focusing on the role of the hypothalamic circuits and other structures that are not completely protected by the blood-brain barrier (BBB) and can serve as virus entry points.

The hypothalamus is a complex structure located within the ventral part of the diencephalon at the base of the brain. It is composed of strongly interconnected cell groups and neuronal circuits. Aside from complex intrahypothalamic connections, the hypothalamus also projects to different brain regions, forming large neuronal networks. The hypothalamic nuclei are involved in the regulation of diverse physiological functions like respiration, the integration of stress response, thermoregulation, cardiovascular and neuroendocrine regulation, glycemia, and consciousness.

One of the main responses of the hypothalamus to various homeostatic challenges is the stress response. It has been suggested that SARS-CoV and SARS-CoV-2 can alter the stress response and cortisol dynamics. The autopsies of individuals infected with SARS-CoV showed a degeneration and necrosis of the adrenal cortical cells. Hypocortisolism and low levels of dehydroepiandrosterone sulfate found in patients infected with SARS-CoV-2 indicate damage to the hypothalamic-pituitary circuits. A recent study has shown that persistent hypotestosteronemia in patients suffering from COVID-19 or long COVID syndrome could be of hypothalamic origin due to impaired gonadotropin-releasing hormone function, or hypogonadotropic hypogonadism.
https://discovermednews.com/hypotestosteronemia-men-covid-19-post-covid-hypothalamic-origin/

Viral entry into the CNS

In the introduction to this article, the authors discussed different pathways for viral entry into the CNS. The neuroinvasion by CoVs 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 BBB, blood-cerebrospinal fluid (CSF) barrier, retrograde axonal transport, and the use of infected hematopoietic cells as "Trojan horses". As clinical studies failed to detect significant levels of viral RNA in the CSF, the CSF has been questioned as a major transportation route for SARS-CoV-2. It seems that viruses mainly enter the CNS *via* olfactory sensory neurons or peripheral sensory nerves, but they can use the olfactory and the hematogenic pathways simultaneously.

The BBB and blood-CSF barrier are highly complex networks that protect the CNS parenchyma from harmful elements, including viruses. The breakdown of the BBB and blood-CSF barrier, induced by inflammation due to systemic viral infection, facilitates the passage of viruses into the CNS.

Certain viruses can overcome these obstacles by infecting vascular endothelial cells. This facilitates the direct passage of viruses through the BBB and blood-CSF barrier to the CNS. However, some CNS structures, like the choroid plexus and the circumventricular

organs, including the hypothalamus, are not completely protected by the BBB and can serve as virus entry points. The absence of the BBB in the median eminence- an adaptation that is essential for peptide neurohormones such as gonadotropin-releasing hormone to reach their target cells in the pituitary gland and for circulating peripheral signals to enter the brain- may represent a gap in the brain's defense mechanisms against pathogens.

The significance of hypothalamic-olfactory system crosstalk for viral infection

Upon receiving extensive peripheral sensory inputs from diverse sources, including the olfactory systems, the hypothalamus, as a relay station, communicates with almost all regions of the brain, especially the brainstem. The connections of the hypothalamus with the olfactory bulb are complex; four areas in the posterior lateral hypothalamus (anterior olfactory nucleus, olfactory tubercle, piriform cortex, and anterior cortical nucleus of amygdala) receive input from the olfactory bulb. Several neuropeptides, including gonadotropin-releasing hormone, neuropeptide Y, leptin, adiponectin, and orexins, modulate connections between the hypothalamus and the olfactory bulb.

The olfactory bulb is thought to be the main gateway for viral entry into the brain. Studies have found that the olfactory bulb is the site of viral entry into the CNS after intranasal inoculation. The viral antigens were detected in CNS regions that possess first- or second-order neural connections with the olfactory bulb, such as the cerebral cortex, basal ganglia, the midbrain, and the hypothalamus. In an animal model, the inoculation of the S1 subunit of the SARS-CoV-2 S protein in the olfactory cavity resulted in increased apoptosis of the olfactory system, brain inflammation, and reduced ACh levels. https://discovermednews.com/s1-protein-causes-brain-inflammation-and-decreases-the-acetylcholine-levels/ Similarly, the intranasal infection of mice with SARS-CoV-2 led to viral positivity in various parts of the eye, including the retina, and elevated viral titers in the brain and lungs. https://discovermednews.com/retinal-inflammation-after-intranasal-infection-with-sars-cov-2/

According to the authors, the olfactory system plays a crucial role in the transport of SARS-CoV-2 into the CNS *via* the hypothalamus.

The role of hypothalamic circuits in the viral infection

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. In the circumventricular organs, the endothelium is fenestrated.

The median eminence of the hypothalamus is such circumventricular organ. It is located in the basal hypothalamus, ventral to the third ventricle, and adjacent to the arcuate nucleus. The median eminence contains a rich capillary plexus, a fenestrated endothelium, and tanycytes, which form the specialized ependymoglial cellular sheet that covers the floor and the basolateral walls of the third ventricle.

It is believed that one of the functions of these cells is to create a barrier that prevents substances in the portal capillary spaces from entering the brain. The unique structure of the BBB at the median eminence/arcuate nucleus interface has a major role in the exposure of hypothalamic neurons to systemic factors, including viruses.

An immunocytochemistry study, which examined the distribution of tight junction proteins in the cells that line the ventricular wall of the third ventricle, revealed a distinct pattern of tight junction protein expression in different locations. The results showed that tanycytes of the median eminence were joined at their apices by functional tight junctions, which is consistent with their role in the regulation of blood-hypothalamus exchange by forming a cellular sheet impermeable to blood-borne molecules at the floor of the third ventricle. In contrast, tanycytes of the arcuate nucleus created a permeable layer that allowed unrestrained diffusion of molecules between the CSF and the arcuate nucleus. These findings confirmed the role of the median eminence/arcuate nucleus interface in the exposure of hypothalamic neurons to systemic factors, including viruses.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2892518/

A recent autopsy study examined possible SARS-CoV-2 infection of hypothalamic neurons and olfactory epithelia in four patients who died of COVID-19. SARS-CoV-2 N proteins were found in the hypothalamus of three COVID-19 patients, including one who had a viremia at the time of death. Abundant SARS-CoV-2 N protein and double-stranded RNA were detected in the median eminence/infundibular nucleus, indicating robust viral entry and replication. Viral double-stranded RNA was also found in olfactory bulb cells bordering the olfactory nerve layer.

On the other hand, the SARS-CoV-2 S protein was found in vessels, some neuron-like cells, and cells of the ependymal wall. Tanycytes were infected, and the S protein level was extremely high in tanycytic endfeets that coexpressed angiotensin-converting enzyme 2 (ACE2) and transmembrane protease, serine 2 (TMPRSS2). Abundant SARS-CoV-2 S protein, ACE2, and TMPRSS2 were also detected in the olfactory nerve. In four patients who died of COVID-19, one-third of the gonadotropin-releasing hormone neurons displayed a

bloated or abnormal morphology.
https://discovermednews.com/hypotestosteronemia-men-covid-19-post-covid-hypothalamic-origin/

Conclusion

The authors emphasized the role of hypothalamic circuits in the SARS-CoV and SARS-CoV-2 infections. They concluded that hypothalamic circuits have a central role in most of the neurological symptoms associated with COVID-19.

Further investigations are needed to identify the precise hypothalamic circuits that are viral entry points and contribute to the neurologic manifestations associated with COVID-19.

This article was published in Viruses.

Journal Reference

Mussa, B.M, Srivastava, A., Verberne, AJM. COVID-19 and Neurological Impairment: Hypothalamic Circuits and Beyond. Viruses 2021, 13, 498. (Open Access)
https://doi.org/10.3390/v13030498