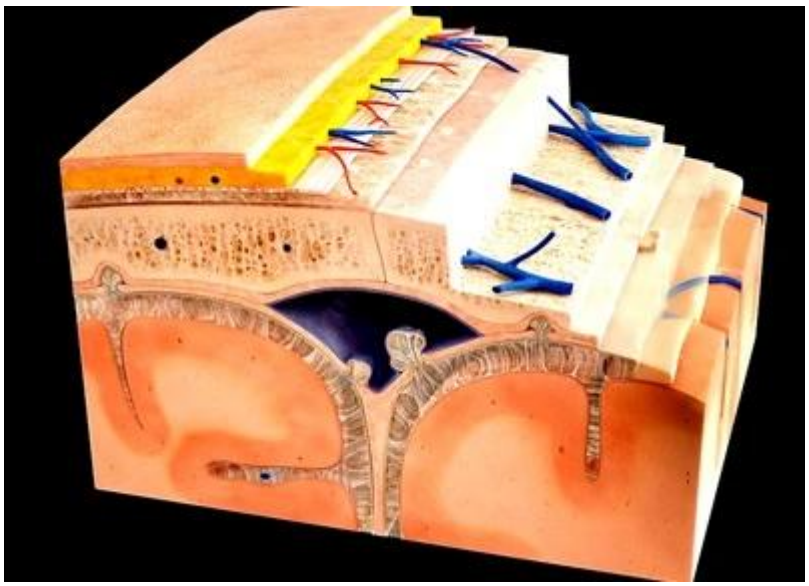
Numerous studies have analyzed the central nervous system (CNS) involvement in COVID-19. To investigate the presence of SARS-CoV-2 in the skull-meninges-brain axis, a consortium of authors, led by scientists from Germany, used mice intravenously injected with fluorescently labeled SARS-CoV-2 S1 unit, and *postmortem* tissue samples from COVID-19 convalescents who died from other causes long after COVID-19 infection.

SARS-CoV-2 is an enveloped, positive-sense, single-stranded RNA virus. Its genome encodes four structural proteins, namely the spike (S), envelope (E), nucleocapsid (N), and membrane (M) proteins. The S protein is a glycosylated homotrimer that comprises two subunits: S1, responsible for receptor binding, and S2, which mediates membrane fusion. The S1 and S2 subunits are produced by proteolytic cleavage of the full-length S protein. The S1 subunit may interact with epithelial and endothelial cells independently of the virion.

It seems that SARS-CoV-2 uses various neuroinvasive strategies and pathways to invade the CNS, such as infection of the nasal olfactory epithelium and axonal transport along the olfactory nerve, retrograde axonal transport, invasion by compromising the blood-brain barrier (BBB), and the use of infected hematopoietic cells as “Trojan horses” (hematogenous route).



The skull bone marrow is characterized as a reservoir for myeloid cells in the channels between the skull marrow and meninges. Also, the CNS border meninges were found to contain a lymphopoietic niche. This suggests that cell reservoirs localized in the skull and

meninges are involved in the skull-meninges-brain axis and, consequently, in various neurological diseases. A recent study revealed an unrecognized role of the bone marrow, as the primary site of hematopoiesis, in multiple sclerosis, promoting intimate interactions between autoreactive T-cells that migrate into the bone marrow and hematopoietic stem cells and progenitor cells.
https://discovermednews.com/autoreactive-t-cells-in-the-bone-marrow-of-ms-patients/

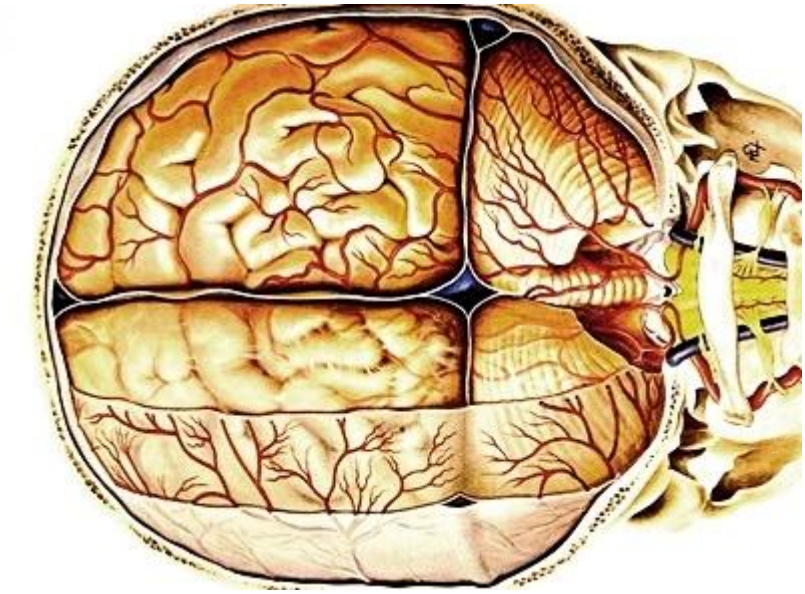
About the Study and Results

After intravenously injecting fluorescently labeled SARS-CoV-2 S1 protein into mice, the S1 protein was detected in all tissues targeted by the virus and accumulated in the skull marrow niches, skull-meninges connection, meninges, and the brain parenchyma after 30 minutes. Importantly, co-staining for vessel visualization showed that the S1 protein was mostly localized in the brain blood vessels.

The S1 protein was also detected in most organs; high concentrations were found close to the liver, kidney, and lung blood vessels, in the testis, ovary, spleen, and bone marrow of other bones, such as the tibia and femur.

The intravenous injection of the S1 protein into mice also caused broad proteome changes in the skull marrow, meninges, and brain, showing the S1 protein immunogenicity in the absence of other viral components. The results showed dysregulation of proteins involved in the complement and coagulation cascade, neutrophil degranulation, neutrophil extracellular traps (NETs) formation, and PI3K-AKT signaling pathway with involvement of key proteins PI3K (phosphatidylinositol 3-kinase) and Akt (protein kinase B).

A dysregulation of the complement and coagulation pathways was observed in both the skull marrow and the brain.



Postmortem examination of tissue samples from COVID-19 convalescents who died long after COVID-19 from other causes by reverse transcription polymerase chain reaction (rt-PCR) demonstrated the presence of SARS-CoV-2 RNA and N protein in 8/16 skull samples and 6/12 meningeal samples. The SARS-CoV-2 S protein was present not only in the skull marrow niches, skull-meninges connections, and meninges but also in blood vessels and the vicinity of cortical neurons positive for NeuN protein, localized in nuclei and perinuclear cytoplasm of most neurons in the central nervous system of mammals. By contrast, all samples of the prefrontal cortex were PCR-negative for SARS-CoV-2 N protein.

Conclusion

This study demonstrates the presence of SARS-CoV-2 S protein in the skull-meninges-brain axis in mice intravenously injected with fluorescently labeled SARS-CoV-2 S1 and human *postmortem* skull samples from COVID-19 convalescents. Of note, human brain samples were PCR-negative for SARS-CoV-2 N protein. The presence of the S1 protein in the mouse skull marrow triggered a broad proteomic change in the skull marrow, meninges, and brain.

According to the authors, the connection between the skull marrow and meninges very likely contributes to the presence of the S1 protein in the brain and meninges in the absence of viral load. This suggests that the S1 protein is a residue from a previous brain infection, or that the highly immunogenic S1 protein from the COVID-19 vaccine triggers infection-independent effects.

The findings of this study showed an alternative route for SARS-CoV-2 S protein entry into the CNS; it might first reach the skull marrow and meninges before entering the brain.

This study has been published on a preprint server and is currently under review.

Journal Reference

Rong Z, Mai H, Kapoor S, et al. SARS-CoV-2 Spike Protein Accumulation in the Skull-Meninges-Brain Axis: Potential Implications for Long-Term Neurological Complications in post-COVID-19. bioRxiv preprint. https://doi.org/10.1101/2023.04.04.535604