The neurologic sequelae that affect the central nervous system (CNS) and peripheral nervous system (PNS) are found in almost 30% of COVID-19 patients. It seems that severe acute respiratory syndrome coronavirus 2 (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 Japanese authors in this study investigated how the intranasal inoculation of the SARS-CoV-2 S1 protein affects the mouse brain and brain acetylcholine (ACh) levels.

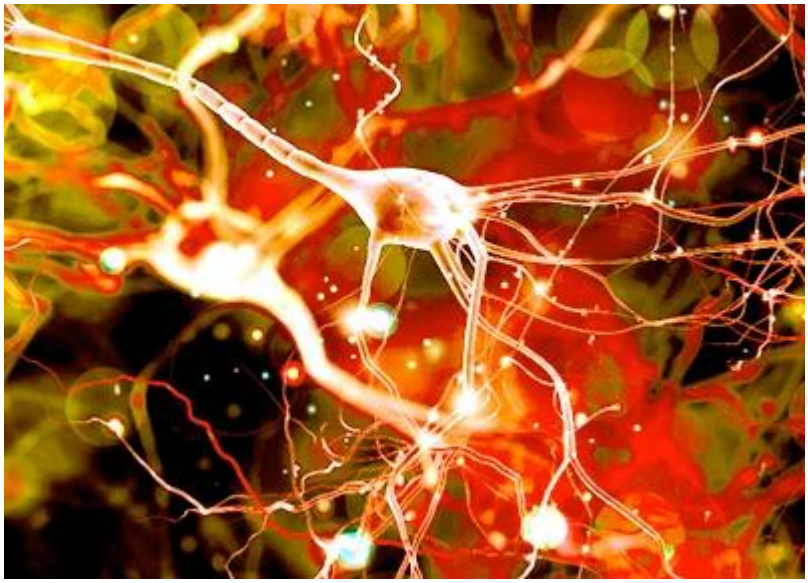
It is assumed that the olfactory bulb serves as the main gateway for SARS-CoV-2 to enter the brain. The cells of the olfactory system express angiotensin-converting enzyme 2 (ACE2) receptor and transmembrane protease, serine 2 (TMPRSS2), which are essential for viral entry. A recent study demonstrated that intranasal infection with SARS-CoV-2 of K18-hACE2 mice resulted in viral positivity in various parts of the eye, including the retina, but also in increased viral titers in the lungs and brain.
https://discovermednews.com/retinal-inflammation-after-intranasal-infection-with-sars-cov-2/

Acetylcholine receptors (AChRs) are classified into either metabotropic muscarinic (mAChRs) or ionotropic nicotinic acetylcholine receptors (nAChRs). Pentameric nAChRs are essential for interneuronal communication within the CNS and the autonomic nervous system. They consist of a varying, either homomeric or heteromeric, combination of nine (α2-α10) α subunits and/or three (β2-β4) β subunits. The activation of nAChR leads to fast and nonselective opening of membrane-bound, excitatory cation channels. ACh is involved in an anti-inflammatory response called the cholinergic anti-inflammatory pathway (CAP) that suppresses inflammation in the brain and peripheral tissues *via* the autonomic nerve fibers.

The SARS-CoV-2 S glycoprotein is composed of the S1 and S2 subunits, separated by host cell proteases. S1 is composed of the N-terminal domain (NTD), the receptor binding domain (RBD) with a receptor binding motif (RBM), and two C-terminal domains. The S protein contains a neurotoxin-like region, located at the junction of the S1 and S2 subunits, with sequence similarities with three *Rabies lyssavirus* (formerly Rabies virus) strains and snake neurotoxins (α-bungarotoxin from snake *Bungarus* genera). Changeux initially suggested that the neurotoxin-like region of the SARS-CoV-2 S glycoprotein interacts with the α-subunits of the nicotinic acetylcholine receptors (nAChRs).
https://comptes-rendus.academie-sciences.fr/biologies/item/CRBIOL_2020__343_1_33_0/

A recent study that investigated whether the neurotoxin-like region of the SARS-CoV-2 S

protein targets nicotinic acetylcholine receptors (nAChRs) has shown that α7nAChR is a target for the neurotoxin-like region of the SARS-CoV-2. The most common nAChRs in the CNS, α4β2 and the α7 subtypes are linked to hyperactivity, aggression, and anxiety. Therefore, understanding how the SARS-CoV-2 neurotoxin-like region affects different nAChR subtypes and associated isoforms provides an understanding of COVID-19 pathophysiology.
https://discovermednews.com/sars-cov-2-and-nicotinic-acetylcholine-receptors/



About the study

The authors created a mouse model that expresses the S1 subunit of the SARS-CoV-2 S protein in the olfactory cavity. In brief, they produced a non-proliferative adenovirus vector expressing the S1 protein (S1 Adv) of the original Wuhan strain and generated the S1 mouse by inoculating the S1 Adv into the nasal cavity.

The behavioral experiments (weight-loaded forced swim test and the tail suspension test) were performed one week after nasal inoculation of the S1 subunit.

To investigate the relationship between systemic and brain inflammation, the scientists enhanced systemic inflammation by intraperitoneal injection of lipopolysaccharide into the S1 mice.

Results

The behavioral experiments demonstrated depressive symptoms in the mice expressing the

S1 protein in their nasal cavity (S1 mouse) manifested as increased fatigue at the weight-loaded forced swim test and increased immobility at the tail suspension test.

The histopathological examination of the whole brain, excluding the olfactory bulb, showed enhanced expression of proinflammatory cytokines (interleukin-6 and tumor necrosis factor-α), and proinflammatory chemokine CCL-2, indicating brain inflammation. The histopathological examination of the olfactory bulb demonstrated enhanced apoptosis.

Cells positive for the ACh-synthesizing enzyme choline acetyltransferase (ChAT) were reduced in the medial septal and diagonal bands of Broca, whereas the Ach levels were reduced throughout mice brains.

The administration of donepezil, a central cholinergic agent and acetylcholine esterase inhibitor, starting from the day of S1 inoculation, normalized inflammatory cytokines that were previously elevated (IL-6 and TNFα). Donepezil also mitigated enhanced apoptosis in the olfactory bulb and inhibited the increase of IL-1β production in the amygdala, induced by the administration of S1. However, donepezil did not reverse the decrease in ChAT-positive cells in the medial septal and diagonal bands of Broca. Donepezil also did not reduce the increased production of inflammatory cytokines in the lungs. Administration of a single lower dose of donepezil one week after S1 Adv inoculation reduced the increased expression of IL-6, TNF α, and CCL-2 in the S1 mice brains.

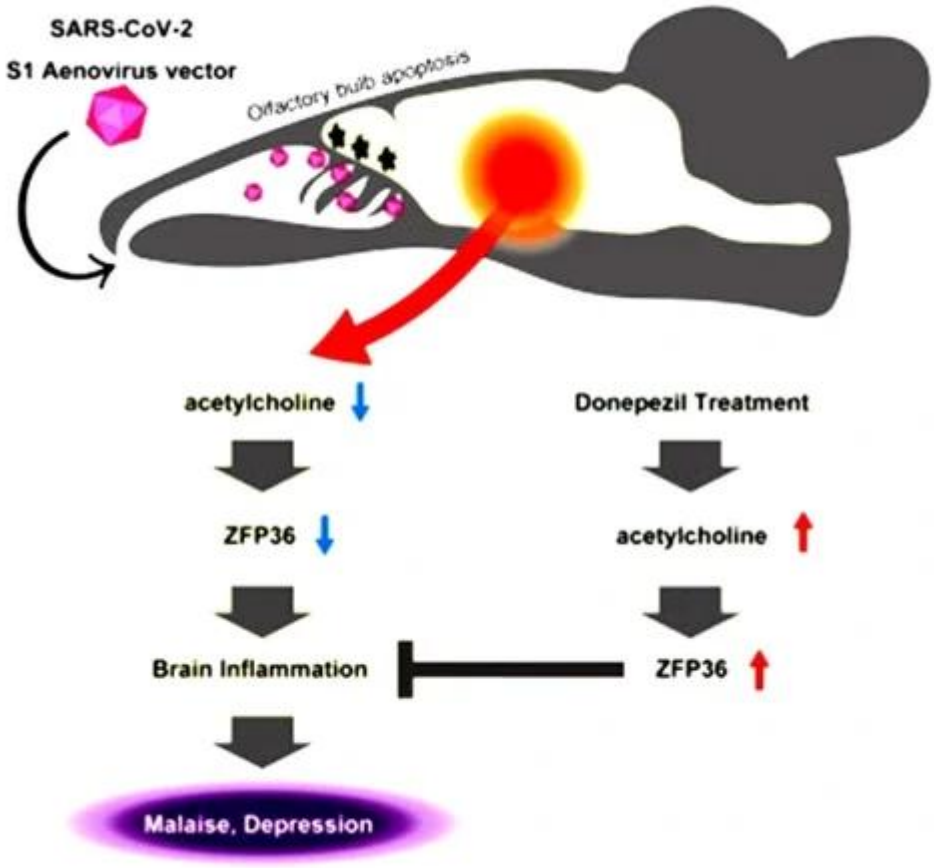


Figure from the original paper by Oka N *et al*.

Since the S1 mRNA was not found in the mouse brain, the authors speculated that the lack of S1 mRNA expression suggests indirect brain inflammation, not caused by direct viral proliferation. In contrast, as S1 mRNA expression was found in the lungs of S1 mice and correlated with the production of inflammatory cytokines, lung inflammation was caused by direct action of the S1 subunit.

In vitro investigation in 3T3 mouse cells and human A549 cells showed intracellular calcium-increasing activity of the NTD on the S1 subunit.

Intracerebroventricular administration of PNU282987, an agonist of α7 nAchRs, was used a week after the S1 inoculation to assess the anti-inflammatory response called the cholinergic anti-inflammatory pathway (CAP). One hour after administration of PNU282987, the expression of inflammatory cytokines in the brains of S1 mice was normalized or

increased. This shows that a disrupted CAP, which suppresses inflammation in the brain and peripheral tissues *via* the autonomic nerve fibers, caused brain inflammation, and that α7 nAchRs are involved in CAP.

The enhancement of systemic inflammation by intraperitoneal injection of lipopolysaccharide into the S1 mice showed markedly reduced expression of calbindin, a marker of γ-aminobutyric acid (GABA)-ergic neurons in mouse brains, and the absence of changes in other markers of neuronal differentiation. The authors interpreted this finding as specific to GABA-ergic neurons.

These results are partly consistent with *in vivo* study by Rhea EM *et al* who explored whether the radioiodinated S1 subunit of the SARS-CoV-2 S protein (I-S1) can cross the BBB after intravenous and intranasal administration in male mice. After intravenous administration, radiolabeled S1 readily crossed the BBB and entered brain regions and the parenchymal space. A dissection of the whole brain into 10 regions showed that I-S1 entered all brain regions, with no statistically significant differences. The enhancement of systemic inflammation by lipopolysaccharide injection increased the amount of I-S1 that entered the brain. The authors speculated that this increase was due to the disruption of the BBB. After intranasal administration, I-S1 was found in all brain regions, but I-S1 levels in the olfactory bulb and hypothalamus were higher than in other brain regions. However, whole-brain I-S1 level, expressed as a percentage of the administered dose, was approximately 10 times higher after intravenous injection than after intranasal administration. https://doi.org/10.1038/s41593-020-00771-8

Conclusion

This study showed that intranasal inoculation of SARS-CoV-2 S1 protein affects the mouse brain resulting in increased apoptosis of the olfactory system, brain inflammation, and reduced ACh levels. This animal model, similar to encephalopathy in COVID-19 patients, demonstrated that brain inflammation was caused indirectly and not by direct action of SARS-CoV-2.

The administration of donepezil, a central cholinergic agent and acetylcholine esterase inhibitor, normalized inflammatory cytokines that were previously elevated and mitigated enhanced apoptosis in the olfactory bulb. According to the authors, donepezil could be used in the treatment of brain inflammation and neurological complications of COVID-19. However, its usefulness needs to be confirmed in clinical trials.

This investigation also showed a link between the S1 protein, brain inflammation, and

reduced ACh production. These findings could contribute to understanding the pathogenesis of neurological complications associated with COVID-19 and long COVID syndrome.

This article was published in iScience.

Journal Reference

Oka, N, Shimada K, Ishi A, et al. SARS-CoV-2 S1 protein causes brain inflammation by reducing intracerebral acetylcholine production, iScience 26, 106954, June 16, 2023. (Open Access). https://doi.org/10.1016/j.isci.2023.106954.