In the postacute phase of COVID-19, there is an increased risk of neurologic sequelae that affect the central nervous system (CNS), such as anosmia, dizziness, headache, stroke, cognitive and memory disorders, extrapyramidal and movement disorders, mental disorders, and encephalitis or encephalopathy, as well as the peripheral nervous system (PNS), such as sensory disorders, polyneuropathy, Guillain–Barré syndrome, orthostatic intolerance, and syncope. In this study, authors from the United States investigated the susceptibility of PNS sensory neurons (from the trigeminal and lumbosacral dorsal root ganglia) and autonomic sympathetic neurons (from the superior cervical ganglia) to infection after intranasal inoculation of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in experimental animals. They also investigated the neuroinvasion of the spinal cord and specific brain regions (olfactory bulb, cortex, hippocampus, brainstem, and cerebellum), as well as the contribution of neuropilin-1 (NRP-1) to SARS-CoV-2 entry into the neurons.

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 olfactory bulb is assumed to be the main gateway for viruses to enter the brain. However, the authors stated that SARS-CoV-2 very likely uses both olfactory and hematogenous pathways.

Trigeminal nerves provide sensory innervation to the oronasal mucosa and project into the brainstem. Superior cervical ganglia provide sympathetic innervation to the salivary and lacrimal glands and the vasculature of the head and brain, while preganglionic neurons reside in the spinal cord. Consequently, both sensory and sympathetic pathways via the trigeminal and superior cervical ganglia could serve as neural pathways for neuroinvasion.

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 serine protease 2 (TMPRSS2), which cleaves the S protein, allowing this binding to take place. In addition to ACE2, the S protein engages other cell-surface factors as attachment factors that promote viral entry.

About the study

The authors inoculated SARS-CoV-2 intranasally into K18-hACE2 transgenic mice (hACE2 mice), wild-type C57BL/6J mice (WT), and golden Syrian hamsters (WT mice and golden Syrian hamsters are hACE2-independent animals). The animals were monitored daily. Death

occurred six days after the infection, and tissue samples were collected.

The authors assessed the susceptibility of PNS sensory neurons (from the trigeminal and lumbosacral dorsal root ganglia) and autonomic sympathetic neurons (from the superior cervical ganglia) to infection with SARS-CoV-2 after intranasal inoculation. They also assessed the neuroinvasion of the spinal cord and specific brain regions (olfactory bulb, cortex, hippocampus, brainstem, and cerebellum), and the contribution of NRP-1 to neuronal entry of SARS-CoV-2.

Results

The presence of viral RNA and specific antigens in neurons of the PNS

The SARS-CoV-2 RNA was detected in neurons of the trigeminal ganglia in both hACE2 and WT mice, and its concentrations were comparable to those observed in neurons of the lumbosacral dorsal root ganglia. The increase in viral RNA concentrations over time suggested genome replication, which was confirmed by double-stranded RNA immunostaining.

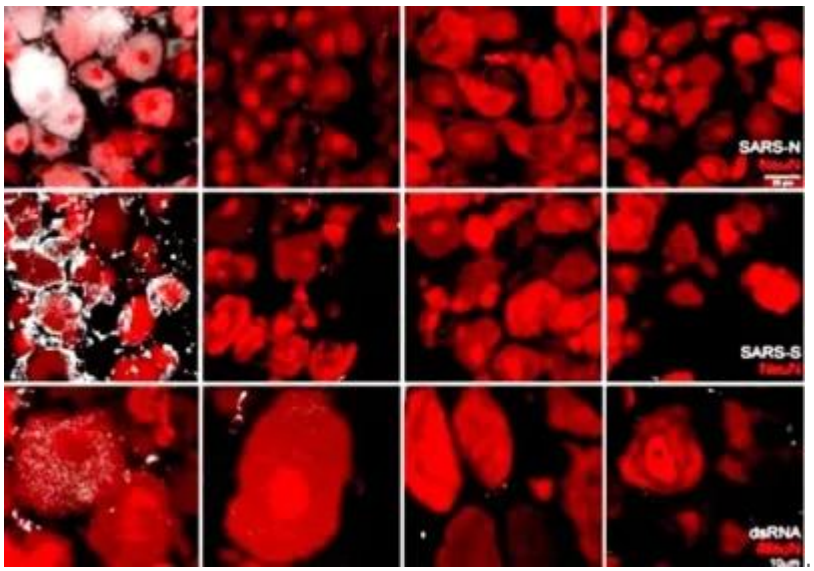

Figure from the original article by Joyce JD, et al. Int J Mol Sci 2024;25: 8245.

The SARS-CoV-2 nucleocapsid (N) protein was detected by immunostaining in 41% of the trigeminal ganglia neurons, 42% of the lumbosacral dorsal root ganglia neurons, and 97% of the superior cervical ganglia neurons from hACE2 mice, and 37% of the trigeminal ganglia neurons, 24% of the lumbosacral dorsal root ganglia, and 93% of the superior cervical ganglia neurons from WT mice. The SARS-CoV-2 S protein was detected in the trigeminal and superior cervical ganglia.

Neurons of the trigeminal and superior cervical ganglia from hACE2 mice displayed a significant pathology, characterized by vacuolated neurons and a loss of ganglionic architecture. Neurons of the same ganglia from WT mice (hACE2-independent animals) remained intact.

The presence of viral RNA and specific antigens in neurons of the CNS

The findings of the diffuse N protein signal throughout the spinal cord and punctate N protein staining inside spinal cord neurons indicated the presence of the free virus or at least the N protein. These results confirm the susceptibility of spinal cord neurons to infection by SARS-CoV-2. All mice and hamsters with early spinal cord infections later developed a brainstem infection.

Six days after the intranasal inoculation, most of the neurons in the frontal cortex, lateral preoptic area, visual cortex, thalamus, and nucleus accumbens were positive for the viral-specific N protein. In hACE2 mice, the highest viral RNA concentrations were found in the hippocampus and brainstem. The viral RNA concentrations were similar in all brain regions in hACE2-independent animals, WT mice, and golden Syrian hamsters. According to these results, the viral spread or replication throughout the CNS differed between ACE2-independent and hACE2 mice.

18 hours after the infection, no viral RNA was detected in the blood. However, it was detected in PNS samples, most CNS samples, and in the salivary gland of both hACE2 and WT mice, showing a direct neural invasion independent of viremia.

The role of NRP-1 as a co-receptor for viral entry into neurons

Since WT mice and golden Syrian hamsters were infected despite the absence of hACE2, researchers investigated the role of NRP-1 in SARS-CoV-2 entry into primary sensory

neurons. The Western blot findings confirmed the expression of NRP-1 in neurons of the trigeminal, superior cervical, and lumbosacral dorsal root ganglia from both hACE2 and WT mice.

When lumbosacral dorsal root ganglion neurons were pretreated with a selective NRP-1 antagonist, EG00229, and then infected with SARS-CoV-2, viral RNA levels decreased by 99.8% in neurons of hACE2 mice, and by 86.7% in neurons of WT mice. These results confirmed that NRP-1 can serve as a co-receptor that enhances infection in the presence of hACE2, or as an alternative receptor independent of hACE2.

Conclusion

This study demonstrated that sensory and autonomic neurons in the PNS and CNS are susceptible to productive infection with SARS-CoV-2 following intranasal inoculation with SARS-CoV-2, which occurs through direct neural invasion that precedes viremia. These results confirmed that axonal transport of SARS-CoV-2 and CNS entry preceded viremia and that neuroinvasion occurred *via* peripheral neural pathways.

According to these results, sensory trigeminal neurons with axonal projections to the oronasal epithelium and brainstem, or superior cervical ganglia neurons with synaptic connections to the salivary glands and brainstem, could serve as an alternative route for CNS invasion, independent of hACE2. Also, distal sensory lumbosacral dorsal root ganglia neurons were equally susceptible to infection, regardless of their location. The question of how the virus reaches the distal ganglia remains uncertain.

In addition, the findings revealed that NRP-1 can serve as a co-receptor for SARS-CoV-2 entry into the neurons of the CNS and PNS.

This study has been published in the International Journal of Medical Sciences.

Journal Reference

Joyce, JD, Moore, GA, Goswami, P, et al. SARS-CoV-2 Rapidly Infects Peripheral Sensory and

Autonomic Neurons, Contributing to Central Nervous System Neuroinvasion before Viremia. *Int. J. Mol. Sci.* 2024, *25*, 8245 (Open Access).

https://www.mdpi.com/1422-0067/25/15/8245 https://doi.org/10.3390/ijms25158245