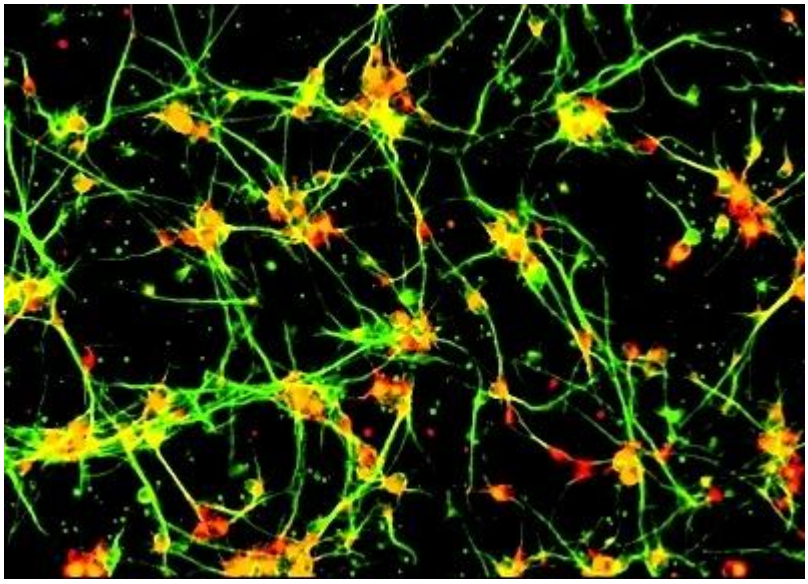
In their previous work, authors from the United States and the Netherlands demonstrated *in vitro* susceptibility and permissivity of dopaminergic (DA) neurons derived from human pluripotent stem cells (hPSCs) to infection with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). By contrast, cortical neurons derived from hPSCs did not show susceptibility to SARS-CoV-2. The same research team in the present article investigated molecular changes in dopaminergic neurons *in vitro* infected with SARS-CoV-2. They also explored whether the changes in DA neurons observed *in vitro* also occur *in vivo,* examining autopsy samples of the *substantia nigra* from patients who died from COVID-19.



Dopaminergic neurons

SARS-CoV-2 is an enveloped, positive-sense, single-stranded RNA virus. Its genome encodes four structural proteins: the spike (S), envelope (E), nucleocapsid (N), and membrane (M) proteins. The S protein is a membrane-bound glycoprotein that binds to the membrane-bound angiotensin-converting enzyme 2 (ACE2) in host cells through the receptor-binding domain located in the S1 subunit.

Parkinson's disease is the second most common neurodegenerative disease. It is caused by a reduction in the dopaminergic neurons of the *substantia nigra*, followed by striatal dopamine depletion. Parkinson's disease is classified as one of the α-synucleinopathies

characterized by the misfolding of alpha-synuclein (α-Syn) into pathological forms, resulting in neurodegeneration. The protein alpha-synuclein is encoded by *SNCA*, the first gene associated with familial Parkinson's disease. Disease is clinically characterized by typical motor symptoms (bradykinesia, rest tremor, and muscle rigidity) and non-motor symptoms (hyposmia, depression, apathy, sleep disorders, and dysautonomia).

Viral infections receive more attention as a cause of viral-induced clinical parkinsonism. However, the specific mechanism of nigrostriatal dopaminergic neuron degradation after viral infection remains unknown. It is worth noting that a study that employed a computational methodology to predict interactions between human and SARS-CoV-2 proteins revealed that SARS-CoV-2 proteins mimic 43 proteins linked to Parkinson's disease pathways, including α-Syn. SARS-COV-2 proteins that possibly interact with α-Syn are NSP7 and 3-CL-like (main) protease. (Yapici-Eser H et al. Neuropsychiatric Symptoms of COVID-19 Explained by SARS-CoV-2 Proteins' Mimicry of Human Protein Interactions. Front. Hum. Neurosci, 23 March 2021)
https://www.frontiersin.org/journals/human-neuroscience/articles/10.3389/fnhum.2021.656313/full

About the Study and Results

Identity of DA neurons was differentiated from hPSCs by immunofluorescence staining with postmitotic DA neuron markers (NURR1: GFP), tyrosine-hydroxylase (TH), MAP2, and FOXA2. Clustering analysis identified four cell populations: three clusters that highly expressed LMO3, a marker of A9 DA neurons, and one cluster that highly expressed CALB1, a marker of A10 DA neurons. According to these results, the population of DA neurons derived from hPSCs was mainly composed of A9 DA neurons, which are a subtype of DA neurons in the *substantia nigra,* mostly affected by Parkinson's disease.

At 24, 48, and 72 hours after SARS-CoV-2 inoculation, the infection of purified DA neurons derived from hPSCs was assessed by quantitative real-time PCR (rt-qPCR) analysis. The results showed that SARS-CoV-2 antigens, including S, E, N, and M proteins, were highly detected in three clusters that expressed a high level of LMO3, a marker of A9 DA neurons (mostly affected by Parkinson's disease in the *substantia nigra)*. The ACE2 blocking prevented SARS-CoV-2 infection of DA neurons derived from hPSCs, suggesting that SARS-CoV-2 infection of DA neurons is ACE2-dependent. In contrast, the SARS-CoV-2 antigens were not detected in a cluster that highly expressed CALB1, a marker of A10 DA neurons.

The infection with SARS-CoV-2 decreased the expression of midbrain DA neuron markers NR4A2, FOXA2, and LMX1A, and in particular, A9 markers LMO3 and DKK3. Quantitative RNA *in situ* hybridization confirmed the loss of A9 DA neurons. According to these results, the A9 DA neuron subtype, which is mostly affected by Parkinson's disease in the *substantia nigra*, was particularly vulnerable to SARS-CoV-2.

Gene analysis revealed that the top-upregulated pathways in SARS-CoV-2-infected DA neurons were the cell cycle, DNA replication, chemokine/cytokine transcripts, inflammation, and senescence pathways. Further analysis confirmed that SARS-CoV-2 triggered cellular senescence in DA neurons and upregulated senescence-associated genes, including CCL2, CCL20, CSF1, CXCL11, GDF15, IGF2R, IL1B, IL6ST, IQGAP1, and TNFRSF11B. The SARS-CoV-2 also upregulated other senescence-associated markers in DA neurons, such as lysosomal senescence-associated β-galactosidase (SA-β-gal) and lipofuscin. DA neurons infected with SARS-CoV-2 displayed senescence-associated phenotypes, including increased lysosome accumulation, mitochondrial dysfunction, and protein oxidation.

By contrast, senescence-associated genes were not upregulated in cortical neurons derived from hPSCs. In addition, the senescence pathway was not upregulated in lung organoids, pancreatic cells, liver organoids, and cardiomyocytes infected with SARS-CoV-2.

It is worth noting that drugs riluzole, metformin, and imatinib, approved by the FDA Food and Drug Administration (FDA), were found to block the senescence of DA neurons mediated by SARS-CoV-2. Riluzole, metformin, and imatinib reduced β-gal activity in a dose-dependent manner without cytotoxicity, down-regulated the genes involved in the senescence pathway, and reduced viral RNA in DA neurons infected with SARS-CoV-2.

Finally, the authors examined the autopsy samples of *substantia nigra* from six patients who died from COVID-19 and three age-matched controls to investigate whether the *in vitro* finding of selective vulnerability of hPSC-derived DA neurons was reflected in the brains of COVID-19 patients. The SARS-CoV-2 transcripts were detected in all six *substantia nigra* samples. Low viral RNA levels were also identified in frozen tissue samples from other brain regions.

Remarkably, the same transcriptional signatures identified in DA neurons infected with SARS-CoV-2 *in vitro* were found in autopsy samples of the *substantia nigra*, such as induction of chemokines/cytokines, inflammation, and senescence-associated genes.

Conclusion

This *in vitro* study demonstrated the selective vulnerability of the A9 DA neuron subtype derived from hPSCs (A9 DA neurons are mostly affected by Parkinson's disease in the *substantia nigra)* to SARS-CoV-2 infection and the associated inflammatory and cellular senescence responses. The comparable inflammatory and senescence signatures in the autopsy samples of the *substantia nigra* suggest that these results may have clinical relevance. However, the authors emphasized the possibility that other cell types, such as astrocytes or microglia, or other pathological changes, such as a hypoxic state, could contribute to the inflammatory and senescence signatures in the *substantia nigra* autopsy samples. They recommended that COVID-19 patients should be closely monitored for an increased risk of developing symptoms related to Parkinson's disease.

This article was published in Cell Stem Cell.

Journal Reference

Yang, L., Kim, TW, Han, Y. et al. SARS-CoV-2 infection causes dopaminergic neuron senescence. Cell Stem Cell 2024; 31, 1–16.
https://www.cell.com/cell-stem-cell/fulltext/S1934-5909(23)00442-3