



Post-viral effects of COVID-19 in the olfactory system and their implications

Michael S Xydakis, Mark W Albers, Eric H Holbrook, Dina M Lyon, Robert Y Shih, Johannes A Frasnelli, Axel Pagenstecher, Alexandra Kupke, Lynn W Enquist*, Stanley Perlman*

Summary

Background The mechanisms by which any upper respiratory virus, including SARS-CoV-2, impairs chemosensory function are not known. COVID-19 is frequently associated with olfactory dysfunction after viral infection, which provides a research opportunity to evaluate the natural course of this neurological finding. Clinical trials and prospective and histological studies of new-onset post-viral olfactory dysfunction have been limited by small sample sizes and a paucity of advanced neuroimaging data and neuropathological samples. Although data from neuropathological specimens are now available, neuroimaging of the olfactory system during the acute phase of infection is still rare due to infection control concerns and critical illness and represents a substantial gap in knowledge.

Recent developments The active replication of SARS-CoV-2 within the brain parenchyma (ie, in neurons and glia) has not been proven. Nevertheless, post-viral olfactory dysfunction can be viewed as a focal neurological deficit in patients with COVID-19. Evidence is also sparse for a direct causal relation between SARS-CoV-2 infection and abnormal brain findings at autopsy, and for trans-synaptic spread of the virus from the olfactory epithelium to the olfactory bulb. Taken together, clinical, radiological, histological, ultrastructural, and molecular data implicate inflammation, with or without infection, in either the olfactory epithelium, the olfactory bulb, or both. This inflammation leads to persistent olfactory deficits in a subset of people who have recovered from COVID-19. Neuroimaging has revealed localised inflammation in intracranial olfactory structures. To date, histopathological, ultrastructural, and molecular evidence does not suggest that SARS-CoV-2 is an obligate neuropathogen.

Where next? The prevalence of CNS and olfactory bulb pathosis in patients with COVID-19 is not known. We postulate that, in people who have recovered from COVID-19, a chronic, recrudescing, or permanent olfactory deficit could be prognostic for an increased likelihood of neurological sequelae or neurodegenerative disorders in the long term. An inflammatory stimulus from the nasal olfactory epithelium to the olfactory bulbs and connected brain regions might accelerate pathological processes and symptomatic progression of neurodegenerative disease. Persistent olfactory impairment with or without perceptual distortions (ie, parosmias or phantosmias) after SARS-CoV-2 infection could, therefore, serve as a marker to identify people with an increased long-term risk of neurological disease.

Introduction

Until 2002, when SARS-CoV crossed the species barrier to infect humans, coronaviruses were considered as minor human pathogens.¹ SARS-CoV and SARS-CoV-2 are related coronaviruses and have 72·8% nucleic acid sequence homology.² Furthermore, both viruses use angiotensin converting enzyme 2 (ACE2) as an entry receptor, which engages the trimeric spike glycoprotein located on the surface of the virion. Despite these similarities, each viral infection has a distinct clinical course.³ Unlike infection with SARS-CoV-2, SARS-CoV infection does not produce olfactory impairment and primarily involves the lower respiratory tract.⁴ This clinical observation is consistent with a long-standing principle in virology: although viral entry receptors and cofactors on the surface of host cells determine infectivity, pathogenesis cannot be inferred from the expression pattern of the viral entry receptor alone.⁵

The neurotropic, neuroinvasive, and neurovirulent features of SARS-CoV-2 are not fully understood. Although sudden-onset anosmia or hyposmia (ie, complete or partial loss of smell) are widely reported to be specific indicators of early infection, the precise manner in which

the olfactory system is impaired has not been fully elucidated.^{6–11} Pooled prevalence estimates reveal olfactory dysfunction in approximately half to three-quarters of people diagnosed with COVID-19, with estimates trending higher when semiobjective quantitative diagnostic tools, which graduate levels of impairment to detect subclinical smell loss, are used.^{12–17}

SARS-CoV-2 is highly pathogenic and possibly infects various cell types and tissues. As a result, SARS-CoV-2 infection causes a range of systemic symptoms.¹⁸ However, it is not clear if symptoms result from direct virus invasion of tissues or from dysregulated and systemic inflammation or widespread microangiopathy (often with resultant microcirculatory thrombi).^{19–22}

Viruses with the intrinsic ability to gain access to neural tissue are fairly uncommon. Neuroinvasiveness can be either facultative and opportunistic (ie, the virus infrequently spreads into off-target cells and tissues) or obligate (ie, the virus replicates within neurons). It is unclear if SARS-CoV-2 strains are explicitly tropic, cytopathic, or both for neural tissue (neurons and glia) or neurovasculature (endothelium).^{19,23} Viral nucleic acid, detected by RT-PCR in neural tissue, might not reflect

Lancet Neurol 2021

Published Online

July 30, 2021

[https://doi.org/10.1016/S1474-4422\(21\)00182-4](https://doi.org/10.1016/S1474-4422(21)00182-4)

[https://doi.org/10.1016/S1474-4422\(21\)00182-4](https://doi.org/10.1016/S1474-4422(21)00182-4)

See Online/Comment/

[https://doi.org/10.1016/S1474-4422\(21\)00202-7](https://doi.org/10.1016/S1474-4422(21)00202-7)

*Contributed equally

Human Performance Wing,

Air Force Research Lab,

US Department of Defense,

Wright-Patterson Air Force

Base, Dayton, OH, USA

(M S Xydakis MD, D M Lyon MS);

Division of Neuroradiology,

Walter Reed National Military

Medical Center, US Department

of Defense, Bethesda, MD, USA

(R Y Shih MD); Massachusetts

General Hospital

(M W Albers MD), Massachusetts

Eye and Ear (E H Holbrook MD),

Harvard Medical School,

Boston, MA, USA; Department

of Anatomy, Université du

Québec à Trois-Rivières,

Trois-Rivières, QC, Canada

(Prof J A Frasnelli MD);

Department of Neuropathology

(Prof A Pagenstecher MD),

Marburg Virology Institute

(A Kupke DVM), Philipps

University of Marburg,

Marburg, Germany; Princeton

Neuroscience Institute,

Princeton University, Princeton,

NJ, USA (Prof L W Enquist PhD);

Department of Microbiology

and Immunology, University of

Iowa, Iowa City, IA, USA

(Prof S Perlman MD)

Correspondence to:

Colonel Michael S Xydakis,

Human Performance Wing,

Air Force Research Lab,

US Department of Defense,

Wright-Patterson Air Force Base,

Dayton, OH 45433, USA

michael.xydakis@gmail.com

Panel 1: Types of olfactory dysfunction after viral infection**Transitory or short-term dysfunction**

- Conductive (obstructive) or mechanical losses (eg, congestion) resulting from blockage of inspired air due to local inflammation and oedema of mucosal tissue in the olfactory cleft and upper nasal passages
- Sensorineural (olfactory epithelium and cranial nerve 1) dysfunction can be subdivided into two types:
 - Altered quantity or function of odorant-binding receptor molecules
 - Neuropraxia or dysfunction of olfactory sensory neurons
- Central (olfactory bulbs and brain) dysfunction could be further subdivided* into:
 - Pathosis isolated to the olfactory bulbs
 - Pathosis isolated to higher-order brain regions such as the piriform cortex and orbitofrontal cortex.

Chronic or permanent dysfunction

- Loss of olfactory epithelium (possibly because of death of neural stem cells)
- Disruption of central olfactory processing networks^{24,25}
 - Uncertain functional recovery

*Top-down effects on central olfactory dysfunction (eg, acute head trauma or Parkinson's disease) are poorly understood.²⁴

direct infection at that site but rather haematogenous spread from distant infected tissues. These knowledge gaps in SARS-CoV-2 tropism and pathogenicity are considerable barriers to understanding the clinical effects of SARS-CoV-2 infection on the olfactory nervous system and CNS.

In this Rapid Review, we discuss the association between post-viral olfactory dysfunction and infection by SARS-CoV-2, summarise the biological pathways, contextualise histological evidence from autopsy studies, and propose a hypothesis about the usefulness of this dysfunction for predicting subsequent neurological disorders. Considering the intertwined relation between smell and taste,^{9,11,15,17} and because little is known about the underlying mechanisms that could account for the complete ageusia (ie, loss of taste) and loss of oral chemesthesis seen alongside post-viral olfactory dysfunction in people with COVID-19, we focus on olfactory symptomatology alone.

Olfactory dysfunction after SARS-CoV-2 infection

The mechanisms underlying olfactory dysfunction in people who have had COVID-19 are difficult to disentangle because of the heterogeneity of presentations (panel 1). Such heterogeneity implies that SARS-CoV-2 infection can impair olfactory function at multiple anatomical levels and through various pathophysiological mechanisms that are not mutually exclusive. The factors underlying differences in recovery are unknown. In most cases of COVID-19,

recovery of olfactory function is rapid, seemingly complete, and typically occurs in parallel with the resolution of physical, sinonasal, and coryzal symptoms. The median time of recovery of function after symptoms of olfactory dysfunction manifest is approximately 10 days, although residual and inapparent hyposmia, along with perceptual distortions, can persist.^{9,11,14,26–29}

In people with COVID-19, endoscopic and radiographic evidence shows that the olfactory clefts of the superior nasal vault are not obstructed, suggesting that hyposmia is not accounted for by the conductive model. However, reversible nasal obstruction of airflow through the superior meatus (so-called olfactory cleft syndrome) is also found in a subset of people with olfactory dysfunction after SARS-CoV-2 infection.^{8,15,30–34}

The recovery rate of olfactory function in people with so-called long COVID (ie, individuals with persistent symptoms for more than 3 months) is still unknown (table). A 12–24 month observation period is required before chronic olfactory impairment can be classified as permanent. Moreover, current studies are generally based on self-reported data rather than a complete rhinological and psychophysical olfactometric examination. Importantly, unlike a cardinal symptom of ongoing infection (eg, fever), continued olfactory impairment does not reflect a contagious state or persistence of SARS-CoV-2 infection.²⁹ In individuals with COVID-19 who have not yet returned to baseline olfactory function, it is unclear whether chronic olfactory impairment is due to irreversible damage of the intranasal primary olfactory neurons embedded in the epithelium of the nasal vault, damage to the olfactory bulb, or dysfunction within other CNS pathways.

Manifestations of central olfactory dysfunction

To the best of our knowledge, no historical data exists on how pathosis confined within the olfactory bulbs (eg, infection and neuroinflammation) manifests clinically, and it is not clear whether pathosis would present as anosmia, perceptual distortions (ie, parosmias or phantosmias), or focal or mild encephalitis.⁴⁴ A local disease process that is isolated to, and contained within, the olfactory bulbs might not produce sufficient characteristic signs and symptoms to enable clinicians to suspect CNS pathosis on clinical grounds alone and, thus, be able to judge these symptoms as being associated with SARS-CoV-2. In addition, acute aseptic encephalitis is a very difficult condition to diagnose, even with clinical, laboratory, and neurodiagnostic findings considered pathognomonic.^{44,45}

A distinctive portrait of short-term and intermediate-term neurological manifestations in survivors of COVID-19 has not yet emerged.⁴⁶ A diverse array of non-specific neurological symptoms (ie, headache, dizziness, fatigue, and dysautonomia) and a COVID-19 diagnosis suggest a causal link, which is often used to suggest neuropathogenicity.⁴⁷ Yet, these vague and ubiquitous

	Prevalence of dysfunction	Follow-up from symptom onset	Country	Assessment method*	Confirmation of infection
Vaira et al ¹¹	29 (21%) of 138 patients	60 days	Italy	Self-report or quantitative olfactometry†	PCR
Andrews et al ¹⁷	60 (68%) of 88 patients	52 days (mean)	Italy and UK	Self-report	PCR
Chiesa-Estomba et al ²⁸	384 (51%) of 751 patients	47 days (mean)	Belgium, France, and Spain	Self-report	PCR or IgG and IgM
Otte et al ³⁵	27 (54%) of 50 patients	49 days	Germany	Quantitative olfactometry	PCR
Carfi et al ³⁶	21 (15%) of 143 patients	60 days (mean)	Italy	Self-report	PCR
Otte et al ³⁷	42 (46%) of 91 patients	58 days (mean)	Germany	Quantitative olfactometry	PCR
Boscolo-Rizzo et al ³⁸	34 (19%) of 183 patients	56 days (mean)	Italy	Self-report	PCR
Klein et al ³⁹	15 (14%) of 105 patients	6 months	Israel	Self-report	PCR
Logue et al ⁴⁰	24 (14%) of 177 patients	169 days (median)	USA	Self-report	PCR
Boscolo-Rizzo et al ⁴¹	87 (60%) of 145 patients	6 months	Italy	Quantitative olfactometry	PCR
Huang et al ⁴²	176 (11%) of 1655 patients	6 months	China	Self-report	PCR
Pilotto et al ⁴³	26 (16%) of 165 patients	6 months	Italy	Self-report	PCR

*Quantitative olfactometry includes either the use of an odour identification test or the Connecticut Chemosensory Clinical Research Center orthonasal olfaction test.
†Quantitative olfactometry in inpatients and self-report in outpatients.

Table: Persistence of olfactory dysfunction beyond 45 days in patients with COVID-19

symptoms often occur in respiratory virus infections and are more likely to be transient disturbances in acute neurological function than signs of a neuropathic disease process (panel 2).^{47,49}

The CNS is protected from infection by intrinsic and innate defence mechanisms. Non-cytolytic antiviral cytokine release by activated glial or infiltrating inflammatory cells is the usual mechanism for blocking viral replication and spread in the CNS. Much research is ongoing about the extent to which neurological symptoms of COVID-19 are due to direct effect on neurons versus maladaptive cytokine deregulation.⁵⁰ At present, evidence showing SARS-CoV-2 infection in the brain or spinal cord is sparse.²² The parainfectious cytokine storm hypothesis⁵⁰ states that post-viral neurological disease is due to unchecked, overexuberant, and sterile immunopathology, with active viral replication playing an initiating but secondary role. Olfactory impairment has not been routinely identified as neurological sequelae of the acute or recovery phases in patients with non-infectious critical-illness-related encephalopathy, a condition that would also be expected to generate innate proinflammatory responses in the brain.⁵¹ Persistent olfactory dysfunction is a feature that is unique to patients with COVID-19 and suggests intrinsic pathosis within olfactory-eloquent intracranial structures, possibly with persistent alterations of primary olfactory neurons.

The mechanisms that underpin loss or perturbation of chemosensory function are unclear, but research is ongoing at a cellular level.^{52–55} Evidence supporting direct viral invasion of olfactory sensory neurons is elusive.⁵² A proposed mechanism for viral invasion involves direct targeting by SARS-CoV-2 of non-neuronal receptor sustentacular support cells, which express the ACE2 receptor and TMPRSS2 (transmembrane protease

Panel 2: Evidence from other respiratory viruses

Many endemic and seasonal respiratory viruses circulate among humans and infect a large part of the world's population. The Global Burden of Disease Study 2015 estimated an incidence of 17.2 billion upper respiratory infections per year (95% uncertainty interval 15.4–19.2 billion).⁴⁸ However, chronic or permanent loss of smell due to infection is rare or inapparent in clinical practice, arguing against widespread neuropathogenicity and permanent olfactory dysfunction for most, if not all, respiratory viruses (including coronaviruses).

Evidence is scant and controversial regarding the viral families that can cause chronic sensory deficits or central olfactory dysfunction. Establishing causality in humans is a challenge, primarily because collection of viral specimens and identification of the pathogen are invasive and complex processes. In addition, people with anosmia typically request medical care long after infection. Evidence on anosmiogenic respiratory viruses and post-viral olfactory dysfunction mostly comes from animal models of infection and historical consensus opinion.

serine 2).⁵³ Once infected and impaired, these cells might disrupt the electrophysiological and biochemical homeostasis of bystander olfactory sensory neurons, and the resultant resource-restricted environment might then silence the olfactory receptor in a manner consistent with transient neuropraxia.⁵³ Other pathophysiological models^{54,55} propose that the local inflammatory response might result in reduced expression or function of cognate odorant-binding receptor molecules expressed on the apical surface of bipolar neurons, leading to impairment of odorant signal transduction.

Evidence from neuroimaging

Conventional MRI in people with COVID-19 has revealed localised abnormalities suggestive of a selective susceptibility of olfactory-eloquent brain regions.^{30,44,56–58} In two separate studies,^{59,60} MRIs were done on medical professionals with anosmia during the acute phase of SARS-CoV-2 infection and severe intracranial bilateral olfactory bulb oedema was found, which had returned to normal on follow-up MRI scans done 24 days⁵⁹ after infection diagnosis and 28 days⁶⁰ after symptom onset. Casez and colleagues⁴⁴ reported hyperintensity of the olfactory tracts on MRI in a patient with anosmia who was positive for SARS-CoV-2 by serological analysis of CSF, which are findings that are consistent with encephalitis. Although the imaging methods were controversial, advanced MRI findings of thickening and clumping of the olfactory fila have been reported,⁶¹ suggesting post-infectious inflammatory neuropathy. Other investigators have reported atrophic changes in the olfactory bulbs on MRI scans in survivors of COVID-19 with persistent post-viral olfactory dysfunction.^{62,63}

Although MRI findings in the olfactory bulbs due to either transcribrial viral inflammation, subviral molecular inflammation, or sterile inflammation can be seen, structural neuroimaging abnormalities cannot be set as the sole criteria for infection. Viral pathosis in the olfactory bulbs might resolve quickly and typically is at or below the level of MRI resolution; therefore, these indicators can be outside the diagnostic reach of routine neuroimaging. Furthermore, in patients with COVID-19, normal structural radiographic morphology does not rule out aberrant functional neuronal electrical activity within the olfactory pathways, as evidenced by abnormal findings on functional MRI and PET-CT scans.^{64,65}

Histopathological and ultrastructural evidence

Collectively, viral studies, clinical symptoms, histopathological and ultrastructural data, and neuroimaging findings do not suggest that SARS-CoV-2 is an obligate neuropathogen.^{18,20–22,46,52–55} In line with clinical data, SARS-CoV-2 might affect the brain indirectly through effects on the cerebral microvasculature, since SARS-CoV-2 is rarely found in endothelial cells.^{52,66,67} The virus might directly or indirectly induce vasculitis,^{20,21} microangiopathy, coagulopathy,^{20,21} and (in rare instances) in-situ circulatory microthrombi,^{20,21,23,52} which could lead to territorial infarctions of neural tissue. Yet, in several autopsy studies,^{66,68–74} no evidence has been found of CNS damage directly attributable to SARS-CoV-2 and no immune cell infiltrates have been found, which challenges the proposed mechanism that SARS-CoV-2 affects the brain indirectly through its effects on CNS neurovasculature. If COVID-19 neurological symptoms are found to be of indirect neurovascular origin or from systemic inflammation, concurrent olfactory dysfunction could be coincidental, rather than correlative, and be the result of an independent site of infection within the olfactory epithelium.

As more CNS histopathology evidence becomes available, the interpretation of reported findings has become highly contested.^{67,75–77} Viruses are unique to other infective pathogens in that their identification mostly occurs via a multipronged testing effort involving detection of diagnostic surrogates (RNA and protein), each with its own technical challenges and limitations. In general, pathologists are more familiar with techniques that culture, stain, and identify bacteria and fungi in tissues than they are with detecting and identifying viruses.

To our knowledge, electron microscopy, often considered the gold standard for viral detection, has only revealed the definitive presence of assembled SARS-CoV-2 virions in the cytoplasm of pneumocytes and, rarely, in alveolar macrophages;^{75,78} assembled SARS-CoV-2 virions might have also been found in endothelial cells^{66,75} and a single case might have been found in an olfactory sensory neuron.⁵² A relevant problem with electron microscopy studies is that various subcellular structures are frequently misidentified as viral particles.^{23,67,76,77,79,80}

Proteomic strategies involving fluorochrome-labelled antibodies directed against the SARS-CoV-2 spike protein or the nucleocapsid protein can reveal the presence of a virus or viral proteins within specific cell types. Yet, in immunohistochemistry, antibody specificity, background reactions, and test–retest reproducibility are major challenges. Appropriate controls are paramount in establishing the specificity of antibodies in immunohistochemistry procedures. Double immunostaining is often necessary to be certain of the cell types infected. There are few immunohistochemistry studies of detectable SARS-CoV-2 proteins or virions in vascular and neuronal cells on autopsy examination.^{52,68,81}

Nucleic acid amplification strategies are the most sensitive tests to detect viral presence. RT-PCR is highly sensitive and specific for determining the presence of not only actively replicating viral genomes but also residual RNA fragments in bodily fluids and tissues. SARS-CoV-2 RNA fragments have been detected in the brain by many investigators.^{20,52,68,74,81–83} Although RT-PCR findings suggest the past presence of virus in the tissue examined, there is no unassailable evidence of intact virions in CNS tissue. Standard RT-PCR results cannot discern which cell types are affected, the specific cellular and spatial localisation within the brain, and whether infectious replication-competent virions were ever in that location. In post-mortem brain tissue, homogenisation of the specimen is required before RT-PCR analysis. As a result, viral genomic material found in any brain region might simply be RNA fragments contained within the cerebral blood vessels that are embedded in the autopsy specimens. These RNA fragments might be within endothelial cells or could have originated at some distant infected tissue from natural shedding during the replication process, with subsequent body-wide haematogenous dissemination.^{20,67,76,82} Viral RNA has not been detected in CNS regions by RNA in-situ hybridisation.^{22,52}

Pathosis within the olfactory bulbs

The spread of virions or subviral ribonucleoprotein complexes might occur through the cribriform plate into the olfactory bulbs of the CNS via a transcellular or a paracellular route (figure), although evidence is sparse and circumstantial (panel 3). Standard haematoxylin and eosin staining has revealed pronounced and preferential inflammation in the olfactory bulbs of some people who have died from COVID-19.^{21,68,73} With standard RT-PCR, the amount of viral RNA has been quantified at autopsy and noted to be in higher concentrations in the olfactory bulbs than in other brain regions.^{52,66,71} By immunohistochemistry, spike glycoprotein has been detected within the parenchyma of the olfactory bulbs in one person who died from COVID-19.⁸¹ In another study of multiple autopsy samples, spike glycoprotein was detected in endothelial cells in the vasculature of the olfactory bulbs.⁵² However, electron microscopy of active and replicative virions and in-situ hybridisation evidence are both absent in the olfactory bulbs. Yet, it is important to note that the olfactory bulbs might also serve as immunosensory effector organs.⁵¹ The olfactory bulbs are crucial in the early and rapid clearing of invading pathogens through this entry-prone interface. Viral clearance is believed to be both rapid and robust, thereby precluding post-mortem identification of virions in people with a lengthy hospital course

and time to autopsy.^{22,52} Microglia and astroglia activation, seen in histological tissue specimens, is consistent with this idea. Moreover, sterile inflammation of the olfactory bulbs, due to fulminant and persistent infection of the subjacent intranasal olfactory receptor, could also be sufficient to either cause or contribute to microglia and astroglia activation (figure).^{22,52,68,84}

Migration of cytokines, chemokines, or (less probably) virions or subviral ribonucleoprotein complexes from infected olfactory epithelial tissue to the olfactory bulbs might also occur through glia transit tubules produced by olfactory ensheathing cells (figure).^{85,86} These specialised cells construct and maintain a lattice of perineural channels or fascicles that envelope and nourish the axons of olfactory sensory neurons and convey regenerating axons through the cribriform plate to the olfactory bulbs. Holbrook and colleagues²⁵ have shown in human beings that, if axons are absent, the fascicles still maintain an open interface to the CSF and the olfactory bulbs. Nanoparticles of environmental pollutants and intranasal nose-to-brain drug delivery systems (which aim to deliver pharmacological agents directly to the CSF) also make use of the olfactory ensheathing cells paracellular pathway.⁸⁷ The rate of diffusion of proinflammatory mediators, viral particles, or ribonucleoprotein complexes in these channels has received little attention and requires additional study.

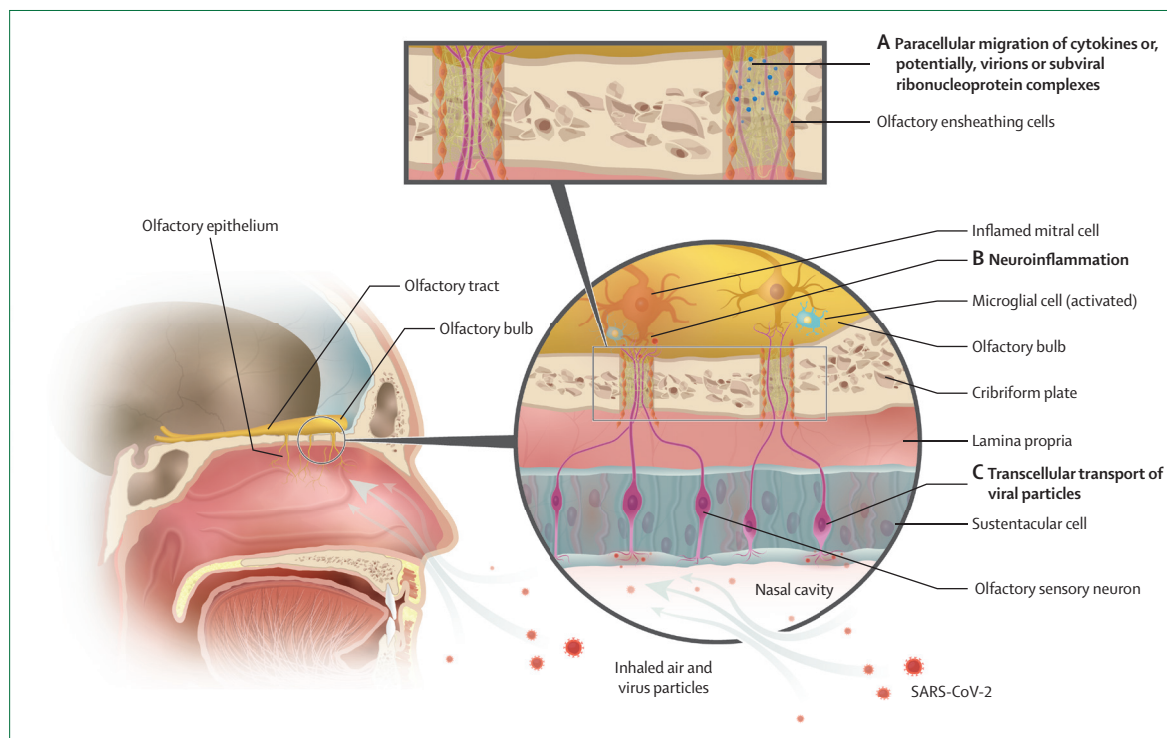


Figure: Potential pathways by which SARS-CoV-2 can infect the olfactory bulbs and generate inflammation

(A) Paracellular migration; molecules or virions can be transported across the cribriform plate through intercellular gaps between the olfactory ensheathing cells or within empty nerve fascicles. (B) Sterile neuroinflammation; immunological response marked by proinflammatory mediators (ie, cytokines and chemokines) that are activated by the virus, which has an initiating but secondary role. (C) The transcellular (trans-synaptic) transport pathway; virions could be transferred across the cribriform plate through anterograde synaptic transport.

See Online for appendix

Panel 3: Viral entry into the CNS via the nose–brain axis

Primary olfactory neurons might be a CNS entry point for viruses through the cribriforme plate. Evidence exists in support of direct neuroinvasion via uptake of virions by olfactory neuronal terminals (located in the nasal epithelium) and retrograde transport to neuronal cell bodies. Invasion of the olfactory bulbs could occur by trans-synaptic spread from olfactory neurons. At the ultrastructural level, evidence from electron microscopy of intact and assembled virions within the olfactory bulbs has been limited to herpes simplex virus type 1, cytomegalovirus, and a single case report of laboratory-acquired rabies (appendix). Human herpesvirus 6 and Borna disease virus have also been detected, but only by molecular signatures and not by electron microscopy. Although influenza A is thought to cause post-viral olfactory dysfunction, only one histopathological case report exists. In this report, virions were visualised in the olfactory bulbs obtained from a child aged 11 months with severe immunodeficiency syndrome.

Within the olfactory epithelium, morphological visualisation of virions has also been restricted to herpes simplex virus type 1, whereas detection of viral proteins and nucleic acids has been reported only in patients with HIV who are immunocompromised (appendix). The scarce histopathological evidence for infection of olfactory epithelium and the olfactory bulbs might be due to the scant availability of human tissues for post-mortem analysis or how uncommon neuroinvasion and neuropathogenicity are in most respiratory viral infections.

In animal models of viral infection, a wide array of human respiratory viruses, including many that are not obligate neuro pathogens, can enter the CNS via the olfactory route after intranasal inoculation. Although laboratory models are useful for characterising some parameters of viral infection, they only partly represent human infections. For example, variables such as viral inoculum dose, innate antimicrobial host tissue defences, adaptive host immune responses, clinical symptoms, and pathological findings are difficult to replicate. Therefore, direct extrapolation from animal experiments to human infections merits caution.

Pathosis in the olfactory epithelium

Although we acknowledge the limitations in data analysis due to the paucity and incompleteness of specimens harvested from patients, active SARS-CoV-2 infection has been directly observed in the olfactory epithelium. Macroscopic and microscopic examination has revealed active inflammation, as seen by the prominent accumulation of immune effector cell infiltrates within the lamina propria and by small areas of focal atrophy of the mucosa.^{52,82,88,89} Molecular testing of the epithelium has revealed subgenomic RNA transcripts, which are often viewed as surrogate markers for active viral replication in a specific location.⁵² Using

Panel 4: Identification of viruses in the nasal cavity

The Human Microbiome Project has identified asymptomatic carriage of various seasonal respiratory viruses, including endemic coronaviruses, within the nasal passages of healthy people (ie, the human virome).⁹¹ These viruses could be symbionts or commensals interacting with the host in various ways, and they appear to coexist alongside actively infecting viruses. Although the human nose transiently harbours respiratory viruses, it would be difficult to distinguish endogenous virome or background viral circulation from a particular agent that might cause post-viral olfactory dysfunction. This difficulty becomes even more problematic when very sensitive nucleic acid amplification techniques are used.⁹² Detection of residual RNA fragments, which often persist within phagocytic cells such as macrophages and desquamating epithelial cells, might not indicate the presence of active viral replication within the nose. For example, the median period when replication-competent SARS-CoV-2 virions can be shed and cultured from the nasal passages of patients who are clinically ill is estimated to be 7–8 days.^{3,93} If standardised sample collection procedures are used, SARS-CoV-2 RNA (detectable by RT-PCR) can persist in asymptomatic carriers, with a median duration of 19 days or 34 days after symptoms onset, according to two separate studies.^{93,94} In addition, even if a virus is identified by use of swabbing in the nasal passages or nasal discharge, identification does not mean that the virus is present within the olfactory epithelium.

antibodies to the spike glycoprotein, viral proteins have also been detected on the apical (external) intranasal surface of the receptor epithelium.^{22,52,90} Using electron microscopy, Meinhardt and colleagues⁵² identified the presence of actively replicating virions within a probable olfactory sensory neuron in one patient who had died from COVID-19. However, these studies do not report any histological evidence of neuronal apoptosis nor any regeneration of olfactory sensory neurons. Histological evidence of widespread denuding and despoiling of the olfactory epithelium is also absent^{22,52,88,90} except in one biopsy study.⁸⁹

Although the intranasal olfactory epithelium is a highly antimicrobial environment, antiviral clearance mechanisms (eg, secretory mucosal immunity) are not analogous to those within the CNS (panel 4). Hence, the olfactory epithelium, rich with sustentacular cells, is probably a region of continuous SARS-CoV-2 replication and viral persistence, enabling a constantly high viral load for either virally mediated or sterile neuroinflammation in the olfactory bulbs.^{22,52}

Conclusions and future directions

After infection with SARS-CoV-2, the olfactory system could be said to serve as a so-called viral sensor, alerting health professionals to the presence of the pathogen.

One benefit of early detection can be the interruption of forward transmission. Currently available radiographic, histological, and molecular data cannot definitively rule out transcribiform, transcellular, or paracellular transit of virions or subviral macromolecules from infected olfactory epithelium to the olfactory bulbs in patients with acute post-viral olfactory dysfunction.⁴⁴ Additionally, immune-mediated olfactory neuropathy and encephalitic damage to the olfactory system accord with residual olfactory dysfunction with or without perceptual distortions (eg, parosmias and phantosmias).⁶⁵ However, these assertions could change as further post-mortem studies are completed and additional histopathological and ultrastructural data, and robust quantitative olfactometric examinations, are published.⁹⁵ Future efforts involving structural and functional MRI of the olfactory system in people with anosmia, done during the acute phase of SARS-CoV-2 infection, would help to close this knowledge gap. Future clinical trials could also be useful to evaluate whether immunomodulatory agents reduce persistent olfactory deficits.

Long-term neurodegenerative sequelae can take years to manifest and might be clinically silent at this early timepoint in the COVID-19 pandemic.^{96,97} Although a definitive link between chronic or permanent olfactory impairment and future neurological vulnerability cannot yet be made, some studies suggest an association.^{40–42,54,55,98,99}

Mounting evidence implicates neuroinflammatory signalling within the brain as a key driver of neurodegenerative diseases. Brain regions involved in processing olfactory input are early sites of the pathological hallmarks of neurodegenerative disease and connect to adjacent brain regions involved in memory and attention.^{97,100} We thus postulate that, in people who have recovered from COVID-19, a chronic or permanent olfactory deficit could be prognostic for an increased likelihood of neurological sequelae or neurodegenerative disorders in the long term. The inflammatory pathways induced by SARS-CoV-2 in the nasal epithelium overlap substantially with inflammatory signalling described in subsets of patients with dementia.^{55,101} An inflammatory stimulus from the nasal epithelium to the olfactory bulbs and connected brain regions might, therefore, accelerate pathological processes and progression of neurodegenerative disease. Although the prevalence of inflammatory signalling in the olfactory bulbs of patients with COVID-19 is unknown, robust inflammation in the nasal olfactory epithelium (as seen in SARS-CoV-2 infections) can propagate sterile inflammation to the olfactory bulbs in animal models.¹⁰¹ Survivors of COVID-19, with or without persistent olfactory impairment, might be at risk of accelerated onset or progression of neurodegenerative disease and should be studied longitudinally with imaging and molecular biomarkers, and cognitive profiling, to test this postulated risk. Additionally, as vaccination efforts reduce mortality, they will also exert an enduring impact on morbidity by decreasing the neurological sequelae of SARS-CoV-2.

Search strategy and selection criteria

We searched PubMed, EBSCO, and Google Scholar from Jan 12, 2020, to May 31, 2021, by combining viral search terms (“SARS-CoV-2”, “COVID-19”, “coronavirus”, “virus”, and “neurotropic”) with olfactory (“olfactory”, “olfaction”, “anosmia”, “smell”, “dysfunction”, “persistence”, and “recovery”) and neuropathology (“ultrastructural”, “neuropathology”, “pathology”, “biopsy”, and “epithelium”) terms. We included publications in any language. We also searched references of relevant articles. Preclinical or in-vitro studies, unless identified as highly translational, were excluded. We supplemented this search with a review of grey literature and preprint servers.

Contributors

MSX, MWA, DML, SP, and LWE planned the Rapid Review. MSX, MWA, EHH, DML, RYS, AP, AK, and JAF contributed to the literature search and article selection. All authors wrote and revised the first draft. MSX, DML, SP, and LWE designed and made the figure. MSX, DML, MWA, LWE, and SP revised the manuscript on the basis of editorial and reviewer comments. All authors reviewed and approved the final draft of the manuscript.

Declaration of interests

SP reports grants from the US National Institutes of Health. AP reports grants from the German Ministry of Education and Research. MWA reports non-financial support from International Flavor & Fragrances, funding from the US National Institutes of Health Rapid Acceleration of Diagnostics (RADx), and a pending COVID-19 smell test patent. All other authors declare no competing interests. The views expressed in this manuscript do not necessarily reflect the official position of the US Department of Defense.

Acknowledgments

We thank Cynthia Goldsmith (Centers for Disease Control and Prevention, Atlanta, GA, USA), Richard Whitley (University of Alabama, Birmingham, AL, USA), Allan Detsky (University of Toronto, Toronto, ON, Canada), and Mary Fowkes (Mount Sinai Hospital, New York, NY, USA) for contributions to the basic, clinical, and neuropathological mechanisms and consequences of viral infections; and Michael G Jordan (Richmond, VA, USA) for figure development.

References

- 1 Ksiazek TG, Erdman D, Goldsmith CS, et al. A novel coronavirus associated with severe acute respiratory syndrome. *N Engl J Med* 2003; **348**: 1953–66.
- 2 Zhou P, Yang XL, Wang XG, et al. A pneumonia outbreak associated with a new coronavirus of probable bat origin. *Nature* 2020; **579**: 270–73.
- 3 Wölfel R, Corman VM, Guggemos W, et al. Virological assessment of hospitalized patients with COVID-2019. *Nature* 2020; **581**: 465–69.
- 4 Booth CM, Matukas LM, Tomlinson GA, et al. Clinical features and short-term outcomes of 144 patients with SARS in the greater Toronto area. *JAMA* 2003; **289**: 2801–09.
- 5 Weiss SR. Forty years with coronaviruses. *J Exp Med* 2020; **217**: e202000537.
- 6 Xydakis MS, Dehghani-Mobaraki P, Holbrook EH, et al. Smell and taste dysfunction in patients with COVID-19. *Lancet Infect Dis* 2020; **20**: 1015–16.
- 7 Speth MM, Singer-Cornelius T, Oberle M, Gengler I, Brockmeier SJ, Sedaghat AR. Olfactory dysfunction and sinonasal symptomatology in COVID-19: prevalence, severity, timing, and associated characteristics. *Otolaryngol Head Neck Surg* 2020; **163**: 114–20.
- 8 Naeini A, Karimi-Galougahi M, Raad N, et al. Paranasal sinuses computed tomography findings in anosmia of COVID-19. *Am J Otolaryngol* 2020; **41**: 102636.

- 9 Cho R, To Z, Yeung Z, et al. COVID-19 viral load in the severity of and recovery from olfactory and gustatory dysfunction. *Laryngoscope* 2020; **130**: 2680–85.
- 10 Payne DC, Smith-Jeffcoat SE, Nowak G, et al. SARS-CoV-2 infections and serologic responses from a sample of US Navy servicemembers—USS Theodore Roosevelt, April 2020. *MMWR Morb Mortal Wkly Rep* 2020; **69**: 714–21.
- 11 Vaira LA, Hopkins C, Petrocelli M, et al. Smell and taste recovery in coronavirus disease 2019 patients: a 60-day objective and prospective study. *J Laryngol Otol* 2020; **134**: 703–09.
- 12 Vaira LA, Hopkins C, Salzano G, et al. Olfactory and gustatory function impairment in COVID-19 patients: Italian objective multicenter-study. *Head Neck* 2020; **42**: 1560–69.
- 13 Yan CH, Faraji F, Prajapati DP, Boone CE, DeConde AS. Association of chemosensory dysfunction and COVID-19 in patients presenting with influenza-like symptoms. *Int Forum Allergy Rhinol* 2020; **10**: 806–13.
- 14 Lechien JR, Chiesa-Estomba CM, Place S, et al. Clinical and epidemiological characteristics of 1420 European patients with mild-to-moderate coronavirus disease 2019. *J Intern Med* 2020; **288**: 335–44.
- 15 Lechien JR, Chiesa-Estomba CM, De Sisti DR, et al. Olfactory and gustatory dysfunctions as a clinical presentation of mild-to-moderate forms of the coronavirus disease (COVID-19): a multicenter European study. *Eur Arch Otorhinolaryngol* 2020; **277**: 2251–61.
- 16 Hannum ME, Ramirez VA, Lipson SJ, et al. Objective sensory testing methods reveal a higher prevalence of olfactory loss in COVID-19 positive patients compared to subjective methods: a systematic review and meta-analysis. *Chem Senses* 2020; **45**: 865–74.
- 17 Andrews P, Pendolino A, Ottaviano G, et al. Olfactory and taste dysfunction among mild-to-moderate symptomatic COVID-19 positive healthcare workers: an international survey. *Investigative Laryngol* 2020; **5**: 1019–28.
- 18 Martinez RB, Ritter JM, Matkovic E, et al. Pathology and pathogenesis of SARS-CoV-2 associated with fatal coronavirus disease, United States. *Emerg Infect Dis* 2020; **26**: 2005–15.
- 19 Varga Z, Flammer AJ, Steiger P, et al. Endothelial cell infection and endotheliitis in COVID-19. *Lancet* 2020; **395**: 1417–18.
- 20 Bryce C, Grimes Z, Pujadas E, et al. Pathophysiology of SARS-CoV-2: targeting of endothelial cells renders a complex disease with thrombotic microangiopathy and aberrant immune response. The Mount Sinai COVID-19 autopsy experience. *medRxiv* 2020; published online May 22. <https://doi.org/10.1101/2020.05.18.20099960> (preprint).
- 21 Lee MH, Perl DP, Nair G, et al. Microvascular injury in the brains of patients with COVID-19. *N Engl J Med* 2021; **384**: 481–83.
- 22 Thakur K, Miller E, Glendinning M, et al. COVID-19 neuropathology at Columbia University Irving Medical Center/ New York Presbyterian Hospital. *Brain* 2021; awab148.
- 23 Paniz-Mondolfi A, Bryce C, Grimes Z, et al. Central nervous system involvement by severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2). *J Med Virol* 2020; **92**: 699–702.
- 24 Xydakis M, Mulligan L, Smith A, Olsen C, Lyon D, Belluscio L. Olfactory impairment and traumatic brain injury in blast-injured combat troops: a cohort study. *Neurology* 2015; **84**: 1559–67.
- 25 Holbrook EH, Leopold DA, Schwob JE. Abnormalities of axon growth in human olfactory mucosa. *Laryngoscope* 2005; **115**: 2144–54.
- 26 Dell'Era V, Farri F, Garzaro G, Gatto M, Aluffi Valletti P, Garzaro M. Smell and taste disorders during COVID-19 outbreak: cross-sectional study on 355 patients. *Head Neck* 2020; **42**: 1591–96.
- 27 Lechner M, Counsell N, Liu J, Eynon-Lewis N, et al. Anosmia and hyposmia in healthcare workers with undiagnosed SARS-CoV-2 infection. *Lancet Microbe* 2020; **1**: e150.
- 28 Chiesa-Estomba C, Lechien J, Radulesco T, et al. Patterns of smell recovery in 751 patients affected by the COVID-19 outbreak. *Eur J Neurol* 2020; **27**: 2318–21.
- 29 Boscolo-Rizzo P, Borsetto D, Fabbris C, et al. Evolution of altered sense of smell or taste in patients with mildly symptomatic COVID-19. *JAMA Otolaryngol Head Neck Surg* 2020; **146**: 729–32.
- 30 Strauss SB, Lantos JE, Heier LA, Shatzkes DR, Phillips CD. Olfactory bulb signal abnormality in patients with COVID-19 who present with neurologic symptoms. *AJNR Am J Neuroradiol* 2020; **41**: 1882–87.
- 31 Lechien J, Michel J, Radulesco T, et al. Clinical and radiological evaluations of COVID-19 patients with anosmia: preliminary report. *Laryngoscope* 2020; **130**: 2526–31.
- 32 Chung T, Sridhar S, Zhang A, et al. Olfactory dysfunction in coronavirus disease 2019 patients: observational cohort study and systematic review. *Open Forum Infect Dis* 2020; **7**: ofaa199.
- 33 Gelardi M, Notargiacomo M, Trecca EMC, Cassano M, Ciprandi G. COVID-19 and nasal cytobrush cytology. *Acta Cytol* 2020; **64**: 397–98.
- 34 Eliezer M, Hamel A, Houdart E, et al. Loss of smell in COVID-19 patients: MRI data reveals a transient edema of the olfactory clefts. *Neurology* 2020; **95**: e3145–52.
- 35 Otte MS, Klussmann JP, Luers JC. Persisting olfactory dysfunction in patients after recovering from COVID-19. *J Infect* 2020; **81**: e58.
- 36 Carfi A, Bernabei R, Landi F. Persistent symptoms in patients after acute COVID-19. *JAMA* 2020; **324**: 603–05.
- 37 Otte MS, Eckel HNC, Poluschkin L, Klussmann JP, Luers JC. Olfactory dysfunction in patients after recovering from COVID-19. *Acta Otolaryngol* 2020; **140**: 1032–35.
- 38 Boscolo-Rizzo P, Polesel J, Spinato G, et al. Predominance of an altered sense of smell or taste among long-lasting symptoms in patients with mildly symptomatic COVID-19. *Rhinology* 2020; **58**: 524–25.
- 39 Klein H, Asseo K, Karni N, et al. Onset, duration, and persistence of taste and smell changes and other COVID-19 symptoms: longitudinal study in Israeli patients. *medRxiv* 2020; published online Oct 17. <https://doi.org/10.1101/2020.09.25.20201343> (preprint).
- 40 Logue JK, Franko NM, McCulloch DJ, et al. Sequelae in adults at 6 months after COVID-19 infection. *JAMA Netw Open* 2021; **4**: e210830.
- 41 Boscolo-Rizzo P, Menegaldo A, Fabbris C, et al. Six-month psychophysical evaluation of olfactory dysfunction in patients with COVID-19. *Chem Senses* 2021; published online Feb 12. <https://doi.org/10.1093/chemse/bjab006>.
- 42 Huang C, Huang L, Wang Y, et al. 6-month consequences of COVID-19 in patients discharged from hospital: a cohort study. *Lancet* 2021; **397**: 220–32.
- 43 Pilotto A, Cristillo V, Piccinelli C, et al. Long-term neurological manifestations of COVID-19: prevalence and predictive factors. *medRxiv* 2021; published online March 14. <https://doi.org/10.1101/2020.12.27.20248903> (preprint).
- 44 Casez O, Willaume G, Grand S, et al. Teaching NeuroImages: SARS-CoV-2-related encephalitis: MRI pattern of olfactory tract involvement. *Neurology* 2021; **96**: e645–46.
- 45 Moriguchi T, Harii N, Goto J, et al. A first case of meningitis/encephalitis associated with SARS-Coronavirus-2. *Int J Infect Dis* 2020; **94**: 55–58.
- 46 Ellul MA, Benjamin L, Singh B, et al. Neurological associations of COVID-19. *Lancet Neurol* 2020; **19**: 767–83.
- 47 Liotta E, Batra A, Clark J, et al. Frequent neurologic manifestations and encephalopathy-associated morbidity in COVID-19 patients. *Ann Clin Transl Neurol* 2020; **7**: 2221–30.
- 48 GBD 2015 Disease and Injury Incidence and Prevalence Collaborators. Global, regional, and national incidence, prevalence, and years lived with disability for 310 diseases and injuries, 1990–2015: a systematic analysis for the Global Burden of Disease Study 2015. *Lancet* 2016; **388**: 1545–1602.
- 49 Helms J, Kremer S, Merdji H, et al. Neurologic features in SARS-CoV-2 infection. *N Engl J Med* 2020; **382**: 2268–70.
- 50 Sinha P, Matthay MA, Calfee CS. Is a “cytokine storm” relevant to COVID-19? *JAMA Intern Med* 2020; **180**: 1152–54.
- 51 Durrant DM, Ghosh S, Klein RS. The olfactory bulb: an immunosensory effector organ during neurotropic viral infections. *ACS Chem Neurosci* 2016; **7**: 464–69.
- 52 Meinhardt J, Radke J, Dittmayer C, et al. Olfactory transmucosal SARS-CoV-2 invasion as a port of central nervous system entry in individuals with COVID-19. *Nat Neurosci* 2020; **24**: 168–175.
- 53 Brann D, Tsukahara T, Weinreb C, et al. Non-neuronal expression of SARS-CoV-2 entry genes in the olfactory system suggests mechanisms underlying COVID-19 associated anosmia. *Sci Adv* 2020; **6**: eabc5801.
- 54 Rodriguez S, Cao L, Rickenbacher G, et al. Innate immune signaling in the olfactory epithelium reduces odorant receptor levels: modeling transient smell loss in COVID-19 patients. *medRxiv* 2020; published online June 16. <https://doi.org/10.1101/2020.06.14.20131128> (preprint).

- 55 Zazhytska M, Kodra A, Hoagland D, et al. Disruption of nuclear architecture as a cause of COVID-19 induced anosmia. *bioRxiv* 2021; published online February 9. <https://doi.org/10.1101/2021.02.09.430314> (preprint).
- 56 Coolen T, Lolli V, Sadeghi N, et al. Early postmortem brain MRI findings in COVID-19 non-survivors. *Neurology* 2020; **95**: e2016–27.
- 57 Lu Y, Li X, Geng D, et al. Cerebral micro-structural changes in COVID-19 patients—an MRI based 3 month follow up study. *EClinicalMedicine* 2020; **25**: 100484.
- 58 Pan S, Chen WC, Baal JD, Sugrue LP. Neuroradiological features of mild and severe SARS-CoV-2 infection. *Acad Radiol* 2020; **27**: 1507–14.
- 59 Laurendon T, Radulesco T, Mugnier J, et al. Bilateral transient olfactory bulb edema during COVID-19-related anosmia. *Neurology* 2020; **95**: 224–25.
- 60 Politi LS, Salsano E, Grimaldi M. Magnetic resonance imaging alteration of the brain in a patient with Coronavirus Disease 2019 (COVID-19) and anosmia. *JAMA Neurol* 2020; **77**: 1028–29.
- 61 Kandemirli S, Altundag A, Yildirim D, Sanli D, Saatci O. Olfactory bulb MRI and paranasal sinus CT findings in persistent COVID-19 anosmia. *Acad Radiol* 2020; **28**: 28–35.
- 62 Chiu A, Fischbein N, Wintermark M, Zaharchuk G, Yun PT, Zeineh M. COVID-19-induced anosmia associated with olfactory bulb atrophy. *Neuroradiology* 2021; **63**: 147–48.
- 63 Tsivgoulis G, Fragkou PC, Lachanis S, et al. Olfactory bulb and mucosa abnormalities in persistent COVID-19-induced anosmia: a magnetic resonance imaging study. *Eur J Neurol* 2021; **28**: e6–8.
- 64 Karimi-Galoungahi M, Yousefi-Koma A, Bakhsayeshkaram M, Raad N, Haseli S. 18FDG PET/CT scan reveals hypoactive orbitofrontal cortex in anosmia of COVID-19. *Acad Radiol* 2020; **27**: 1042–43.
- 65 Ismail I, Gad K. Absent blood oxygen level-dependent functional magnetic resonance imaging activation of the orbitofrontal cortex in a patient with persistent cacosmia and cacogeusia after COVID-19 infection. *JAMA Neurol* 2021; **78**: 609–10.
- 66 Menter T, Haslbauer JD, Nienhold R, et al. Postmortem examination of COVID-19 patients reveals diffuse alveolar damage with severe capillary congestion and variegated findings in lungs and other organs suggesting vascular dysfunction. *Histopathology* 2020; **77**: 198–209.
- 67 Varga Z, Flammer A, Steiger P, et al. Electron microscopy of SARS-CoV-2: a challenging task – Authors' reply. 2020; **395**: e100.
- 68 Matschke J, Lütgehetmann M, Hagel C, et al. Neuropathology of patients with COVID-19 in Germany: a post-mortem case series. *Lancet Neurol* 2020; **19**: 919–29.
- 69 Schaller T, Hirschiühl K, Burkhardt K, et al. Postmortem examination of patients with COVID-19. *JAMA* 2020; **323**: 2518–20.
- 70 Al-Sarraj S, Troakes C, Hanley B, et al. Invited review: the spectrum of neuropathology in COVID-19. *Neuropath Appl Neurobiol* 2020; **47**: 3–16.
- 71 Deigendesch N, Sironi L, Kutza M, et al. Correlates of critical illness-related encephalopathy predominate postmortem COVID-19 neuropathology. *Acta Neuropathol* 2020; **140**: 583–86.
- 72 Jensen M, Quesne J, Officer-Jones L, et al. Neuropathological findings in two patients with fatal COVID-19. *Neuropathol Appl Neurobiol* 2020; **47**: 17–25.
- 73 Schurink B, Roos E, Radonic T, et al. Viral presence and immunopathology in patients with lethal COVID-19: a prospective autopsy cohort. *Lancet Microbe* 2020; **1**: e290–99.
- 74 Solomon IH, Normandin E, Bhattacharyya S, et al. Neuropathological features of COVID-19. *N Engl J Med* 2020; **383**: 989–92.
- 75 Dittmayer C, Meinhardt J, Radbruch H, et al. Why misinterpretation of electron micrographs in SARS-CoV-2-infected tissue goes viral. *Lancet* 2020; **396**: e64–65.
- 76 Hopfer H, Herzig M, Gosert R, et al. Hunting by transmission electron microscopy—a guide to SARS-CoV-2 associated ultrastructural pathology in COVID-19 tissues. *Histopathology* 2020; **78**: 358–70.
- 77 Goldsmith CS, Miller SE, Martinez RB, Bullock HA, Zaki SR. Electron microscopy of SARS-CoV-2: a challenging task. *Lancet* 2020; **395**: e99.
- 78 Carsana L, Sonzogni A, Nasr A, et al. Pulmonary post-mortem findings in a series of COVID-19 cases from northern Italy: a two-centre descriptive study. *Lancet Infect Dis* 2020; **20**: 1135–40.
- 79 Bulfamante G, Chiumello D, Canevini MP, et al. First ultrastructural autopic findings of SARS-Cov-2 in olfactory pathways and brainstem. *Minerva Anestesiol* 2020; **86**: 678–79.
- 80 Morbini P, Benazzo M, Verga L, et al. Ultrastructural evidence of direct viral damage to the olfactory complex in patients testing positive for SARS-CoV-2. *JAMA Otolaryngol Head Neck Surg* 2020; **146**: 972–73.
- 81 Nampothiri S, Sauve F, Ternier G, et al. The hypothalamus as a hub for SARS-CoV-2 brain infection and pathogenesis. *bioRxiv* 2020; published online June 19. <https://doi.org/10.1101/2020.06.08.139329> (preprint).
- 82 Al-Dalahmah O, Thakur KT, Nordvig AS, et al. Neuronophagia and microglial nodules in a SARS-CoV-2 patient with cerebellar hemorrhage. *Acta Neuropathol Commun* 2020; **8**: 147.
- 83 Puelles VG, Lütgehetmann M, Lindenmeyer MT, et al. Multiorgan and renal tropism of SARS-CoV-2. *N Engl J Med* 2020; **383**: 590–92.
- 84 Zhou Y, Song WM, Andhey PS, et al. Human and mouse single-nucleus transcriptomics reveal TREM2-dependent and TREM2-independent cellular responses in Alzheimer's disease. *Nat Med* 2020; **26**: 131–42.
- 85 Kupke A, Becker S, Wewetzer K, Ahlemeyer B, Eickmann M, Herden C. Intranasal Bornavirus (BoDV-1) infection: insights into initial steps and potential contagiousity. *Int J Mol Sci* 2019; **20**: 1318.
- 86 van Riel D, Verdijk R, Kuiken T. The olfactory nerve: a shortcut for influenza and other viral diseases into the central nervous system. *J Pathol* 2015; **235**: 277–87.
- 87 Maher M, Ahmed I, Karloukovski V, et al. Magnetite pollution nanoparticles in the human brain. *Proc Natl Acad Sci USA* 2016; **113**: 10797–801.
- 88 Kirschenbaum D, Imbach LL, Ulrich S, et al. Inflammatory olfactory neuropathy in two patients with COVID-19. *Lancet* 2020; **396**: 166.
- 89 Vaira L, Hopkins C, Sandison A, et al. Olfactory epithelium histopathological findings in long-term coronavirus disease 2019 related anosmia. *J Laryngol Otol* 2020; **134**: 1123–27.
- 90 Cantuti-Castelvetri L, Ojha R, Pedro L, et al. Neuropilin-1 facilitates SARS-CoV-2 cell entry and infectivity. *Science* 2020; **370**: 6518.
- 91 National Institutes of Health. Human Microbiome Project. May 24, 2019. <https://commonfund.nih.gov/hmp/public> (accessed Oct 22, 2020).
- 92 Tian J, Pinto J, Li L, Zhang S, Sun Z, Wei Y. Identification of viruses in patients with postviral olfactory dysfunction by multiplex reverse-transcription polymerase chain reaction. *Laryngoscope* 2020; **131**: 158–64.
- 93 Kim, M, Cui, C, Shin, K, et al. Duration of culturable SARS-CoV-2 in hospitalized patients with COVID-19. *N Engl J Med* 2021; **384**: 671–73.
- 94 Long Q, Tang X, Shi Q, et al. Clinical and immunological assessment of asymptomatic SARS-CoV-2 infections. *Nat Med* 2020; **26**: 1200–04.
- 95 Koralknik IJ, Tyler KL. COVID-19: a global threat to the nervous system. *Ann Neurol* 2020; **88**: 1–11.
- 96 Kohli P, Soler ZM, Nguyen SA, Muus JS, Schlosser RJ. The association between olfaction and depression: a systematic review. *Chem Senses* 2016; **41**: 479–86.
- 97 Xydakis MS, Belluscio L. Detection of neurodegenerative disease using olfaction. *Lancet Neurol* 2017; **16**: 415–16.
- 98 Fragiel M, Miro O, Llorens P, et al. Incidence, clinical, risk factors and outcomes of Guillain-Barré syndrome in patients with Covid-19. *Ann Neurol* 2021; **89**: 589–603.
- 99 Speth M, Singer-Cornelius T, Oberle M, et al. Mood, anxiety and olfactory dysfunction in COVID-19: evidence of central nervous system involvement? *Laryngoscope* 2020; **130**: 2520–25.
- 100 Long JM, Holtzman DM. Alzheimer disease: an update on pathobiology and treatment strategies. *Cell* 2019; **179**: 312–39.
- 101 Rodriguez S, Schrank BR, Sahin A, et al. Genome-encoded cytoplasmic double-stranded RNAs, found in C9ORF72 ALS-FTD brain, propagate neuronal loss. *Sci Transl Med* 2021; **13**: eaaz4699.

Published by Elsevier Ltd.