The nervous system during COVID-19: Caught in the crossfire

Nick R. Natale\textsuperscript{1,2,3,4} | John R. Lukens\textsuperscript{2,3,4,5} | William A. Petri Jr\textsuperscript{1,2,3,4,5,6,7}

\textsuperscript{1}Division of Infectious Diseases and International Health, Department of Medicine, University of Virginia Health System, Charlottesville, Virginia, USA
\textsuperscript{2}Center for Brain Immunology and Glia (BIG), Department of Neuroscience, University of Virginia, Charlottesville, Virginia, USA
\textsuperscript{3}Neuroscience Graduate Program, University of Virginia, Charlottesville, Virginia, USA
\textsuperscript{4}Global Biothreats Graduate Training Program, University of Virginia, Charlottesville, Virginia, USA
\textsuperscript{5}Department of Microbiology, Immunology and Cancer Biology, University of Virginia Health System, Charlottesville, Virginia, USA
\textsuperscript{6}Department of Medicine, University of Virginia School of Medicine, Charlottesville, Virginia, USA
\textsuperscript{7}Department of Pathology, University of Virginia Health System, Charlottesville, Virginia, USA

Correspondence
John R. Lukens, Department of Neuroscience, Center for Brain Immunology and Glia, University of Virginia, 409 Lane Road, MR4-6154, Charlottesville, VA 22908, USA. Email: jrl7n@virginia.edu
William A. Petri, Jr, Department of Medicine, Division of Infectious Diseases, University of Virginia, 345 Crispell Drive, Charlottesville, VA 22908, USA. Email: wap3g@virginia.edu

SUMMARY
SARS-CoV-2, the virus that causes coronavirus disease (COVID)-19, has become a persistent global health threat. Individuals who are symptomatic for COVID-19 frequently exhibit respiratory illness, which is often accompanied by neurological symptoms of anosmia and fatigue. Mounting clinical data also indicate that many COVID-19 patients display long-term neurological disorders postinfection such as cognitive decline, which emphasizes the need to further elucidate the effects of COVID-19 on the central nervous system. In this review article, we summarize an emerging body of literature describing the impact of SARS-CoV-2 infection on central nervous system (CNS) health and highlight important areas of future investigation.

KEYWORDS
anosmia, COVID-19, NeuroCOVID, neuroinflammation, neuroimmunology, olfactory system, SARS-CoV-2

1 | INTRODUCTION

SARS-CoV-2, the virus responsible for the coronavirus disease (COVID)-19 pandemic, poses a constant threat to global human health. Since its first detection in Wuhan, China in 2019,\textsuperscript{1} approximately 500 million documented cases of COVID-19 have been reported around the globe. The explosive spread of SARS-CoV-2 and the failure to contain the virus through isolation policies can be owed to SARS-CoV-2’s efficient airborne transmissibility and its ability to replicate in hosts without generating symptoms. For the majority of COVID-19 cases today, the infected individual is either asymptomatic or experiences mild symptoms consisting of anosmia (loss of smell), fever, fatigue, headache, cough, muscle aches, and loss of appetite.\textsuperscript{2,3} However, for certain vulnerable populations, SARS-CoV-2 infection can spiral into a severe disease that is accompanied by an acute respiratory distress syndrome (ARDS). If left untreated, severe COVID-19 can be deadly, and predictive models estimate that SARS-CoV-2 infection is implicated in 18.2 million deaths worldwide.\textsuperscript{4}

Although SARS-CoV-2 is often described as a respiratory virus, COVID-19 is best described as a multifaceted inflammatory...
syndrome. SARS-CoV-2 and its sarbecovirus relatives are notorious for instigating uncontrolled inflammation in their host, commonly referred to as "cytokine storms." The structural and accessory proteins of SARS-CoV-2 are potent stimulators of the host’s innate immune system, and this overstimulation can lead to chronic inflammation in a variety of organs including the brain. At present, there is mounting evidence that severe COVID-19 can damage the central nervous system (neuro-COVID), which is congruent with the growing public concern that survivors of severe COVID-19 have an increased risk of developing neurological disorders. Following infection, many COVID-19 patients describe experiencing "brain-fog." Emerging clinical data indicate that COVID-19 survivors display postacute [neurological] sequelae of COVID-19 (neuro-PASC) such as prolonged anosmia, depression, memory loss, and cognitive decline.

There is currently a substantial gap in our knowledge of how COVID-19 damages the central nervous system (CNS). It is unclear whether the SARS-CoV-2 virus itself is directly causing damage in the CNS or whether the host's immune response to SARS-CoV-2 infection is inadvertently causing damage in the CNS through inflammatory processes. Therefore, this review will highlight several recent studies that have attempted to elucidate the acute and long-term effects of COVID-19 on the CNS.

2 | BACKGROUND

2.1 | The emergence and evolution of SARS-CoV-2

Due to their high zoonotic potential, coronaviruses frequently overcome species barriers through natural selection in the animal host and sporadically spillover into the global human population. The seven coronaviruses known to infect humans are all within the alpha-coronavirus and beta-coronavirus genera. Viruses belonging to the Sarbecovirus (e.g., SARS-CoV and SARS-CoV-2) or Merbecovirus (e.g., MERS-CoV) subgenera can cause severe disease in humans, while seasonal viruses in the Embeccovirus (e.g., HCoV-OC43 and HCoV-HKU1), Duvina-coronavirus (e.g., HCoV-229E), and Setracovirus (e.g., HCoV-NL63) subgenera often cause mild "common cold" symptoms in humans. Five of the seven human-infecting coronaviruses are hypothesized to be bat-derived, and the transmission of these bat coronaviruses to humans seems to be dependent on an intermediate host (e.g., bovine, camels, camelpids, and civets). Asian horseshoe bats harbor the vast majority of known sarbecoviruses, but sarbecovirus-infected horseshoe bats have also been detected in Slovenia and Kenya. Sarbecoviruses are all derived from a common ancestor that utilizes the mammalian angiotensin-converting enzyme 2 (ACE2) receptor for entry into host cells. Today, SARS-like virus clades in Asia and the BtKY72 virus in Kenya utilize ACE2, while a new HKU3-related sarbecovirus clade has become independent of ACE2. The severe disease associated with sarbecovirus infections in humans does not seem to be related to the exploitation of the ACE2 receptor because the mild HCoV-NL63 alphacoronavirus also binds ACE2.

The emergence of a highly transmissible sarbecovirus with pandemic-potential was not unexpected but anticipated for years. SARS-like sarbecoviruses gained the ability to bind to the ACE2 receptors of two potential intermediate hosts, civets, and rodents, both of which were actively traded in Wuhan wet markets. Moreover, between 2013 and 2019, a crescento of metadata collected by the Wuhan Institute of Virology (WIV) and Dr. Ralph S. Baric and colleagues contended that Asian horseshoe bat-derived SARS-like viruses were already capable of efficiently binding and infecting through the human ACE2 receptor. SARS seropositivity surveillance by WIV in 2018 hints that a SARS-like virus may already have been circulating in villages adjacent to bat-populated caves in the Yunnan province in China, suggesting a regular human exposure to a possible precursor to SARS-CoV-2.

SARS-CoV-2 likely emerged through either bat-to-human transmission or bat-to-pangolin-to-human transmission. The RaTG13 and RpYN06 SARS-like viruses isolated from Chinese horseshoe bats have, respectively, 96.2% and 94.5% nucleotide sequence homology to ancestral SARS-CoV-2, but their ACE2 receptor-binding residues are vastly different from SARS-CoV-2. On the contrary, although pangolin-derived SARS-like sarbecoviruses isolated in the Guandong province of China have only 85.5% to 92.4% nucleotide sequence similarity to ancestral SARS-CoV-2, their receptor-binding domain sequence and structure is nearly identical to ancestral SARS-CoV-2 and can engage human ACE2 with a higher affinity than ancestral SARS-CoV-2. The residues on human ACE2 that are contacted by SARS-CoV-2 are quite different from pangolin ACE2, suggesting that the similarity of receptor-binding domains of pangolin-derived SARS-like viruses and SARS-CoV-2 is not the result of ACE2-driven natural selection but the divergence from a common ancestor.

While the discovery of new bat-derived and pangolin-derived sarbecoviruses has partially filled the gaps in our knowledge of how a SARS-like virus evolved into SARS-CoV-2, the de novo addition of a polybasic site (RRRAR, also referred to as S1/S2) into the SARS-CoV-2 spike protein is peculiar. The presence of a polybasic cleavage site on the virus protein that interacts with the host receptor is the rate-limiting step for whether or not a coronavirus (e.g., MERS-CoV, HCoV-HKU1, and HCoV-OC43) or influenza virus becomes highly transmissible. Two mutations surrounding this polybasic pocket are blamed for the enhanced transmissibility of the SARS-CoV-2 Omicron variant compared to previous variants of concern. Without this polybasic site, SARS-CoV-2 is unable to spread airborne and its disease is attenuated in animal models. Such a polybasic site has never been detected in pangolin-derived sarbecoviruses thus far, which may explain why pangolin-derived sarbecoviruses fail to be transmitted through aerosol and replicate efficiently in other mammalian hosts. Therefore, the natural acquisition of SARS-CoV-2’s polybasic site remains a mystery, and the lack of data on SARS-like sarbecovirus carrying this polybasic site leaves room for conspiracies of SARS-CoV-2 being initially created in a laboratory setting.
The mutation-dependent evolutionary plasticity of SARS-like sarbecoronaviruses binding ACE2 orthologs is remarkable, which explains the high zoonotic capabilities of these viruses. When ancestral SARS-CoV-2 first emerged, it could efficiently bind and infect through the ACE2 receptor of non-human primates, bats, mink, ferrets, deer mice, felines, canines, rabbits, hamsters, and white-tailed deer (Figure 1). As SARS-CoV-2 continues to spread in mammalian populations around the world, the virus has gained mutations in its receptor-binding domain that have augmented its transmissibility and improved its pan-ACE2-binding, while expanding the range of species that it can infect. For example, in 2020, deep mutational scanning of the SARS-CoV-2 binding, while expanding the range of species that it can infect. For example, in 2020, deep mutational scanning of the SARS-CoV-2 receptor-binding domain identified the N501 residue as a constraint for increased pan-ACE2-binding affinity. Soon after, the circulating SARS-CoV-2 virus naturally gained the N501Y mutation; and its aerosol transmission, human ACE2-binding affinity, and ability to infect mice and rats was drastically improved (Figure 1).

SARS-CoV-2 variants of concern, such as Delta and Epsilon, have demonstrated that the virus has mutation flexibility to escape from neutralizing antibodies while also enhance transmissibility and infectivity across multiple species. However, due to selective pressures, it seems that the current dominant SARS-CoV-2 strain (Omicron variant) sacrificed its replication fitness to evade naturally occurring neutralizing antibodies. Despite its enhanced transmissibility and ACE2-binding affinity, the SARS-CoV-2 Omicron variant has attenuated fitness across a variety of human cell lines and animals. This loss of fitness by the SARS-CoV-2 omicron variant, reported herein as “Omicron,” is due to multiple unfavorable mutations surrounding its spike S2’ site. This is a bizarre strategy by Omicron because efficient cleavage of the S2’ site is crucial for the infectivity of ancestral SARS-CoV-2 and the alpha, beta, gamma, and delta variants that preceded Omicron, reported herein as SARS-CoV-2(α-δ). Despite these drastic actions by Omicron to evade immune detection through antigenic shifts, a handful of broad-spectrum neutralizing antibodies and plasma from convalescent and vaccinated individuals can still neutralize Omicron.

As history has shown us, all previous SARS-CoV-2 variants of concern arose to dominance through virus lineages that were separate from the prevailing lineage at the time. Therefore, although the current dominant variant of SARS-CoV-2 has impaired fitness and possibly elicits a less severe disease, we should expect future variants of SARS-CoV-2 to have unpredictable severity and immune evasion capabilities.

2.2 | The structure and life cycle of SARS-CoV-2

SARS-CoV-2 is a positive-sense RNA, enveloped virus that is studied with glycoproteins called spikes that enable the virus to infect cells through engagement of the mammalian host’s angiotensin-converting enzyme 2 (ACE2) receptor. Recent studies suggest that cellular senescence increases the transcription of ACE2 — a critical predilection that may account for severe COVID-19 disproportionately affecting older populations. Unlike SARS-CoV, SARS-CoV-2 displays an important polybasic site on the junction of two spike glycoprotein subunits (S1/S2). Before a newly made SARS-CoV-2 virion undergoes exocytosis from an infected host cell, the mammalian protein furin, a calcium-dependent serine protease on the Golgi apparatus, precleaves this polybasic site. Furin precleavage of the S1/S2 site reduces the need for SARS-CoV-2 to search for proteases on target cells to prime its spike protein for ACE2 attachment. Once a SARS-CoV-2 virion encounters a host cell membrane, co-factors such as heparan sulfate and sialic acid, which are also utilized by herpesviruses, influenza viruses, and other coronaviruses, aid in viral attachment to ACE2.

On the surface of the target cell membrane, SARS-CoV-2(α-δ) relies upon transmembrane protease serine 2 (TMPRSS2) on the target cell to cleave the S2’ site of its spike — a strategy also employed by SARS-CoV and Influenza A. The co-expression of ACE2 and TMPRSS2 makes host cells frequent targets of SARS-CoV-2(α-δ) infection, particularly Type II pneumocytes in the alveolar epithelium, multiciliated cells in the nasal respiratory epithelium, and sustentacular cells in the olfactory neuroepithelium. If the S1/S2 site of...
the SARS-CoV-2 spike was not precleaved by furin, thereby blocking downstream TMPRSS2 cleavage of the S2' site, host cathepsins can cut at sites between S1/S2 and S2' as an alternative to priming the S2 subunit for ACE2 fusion.\textsuperscript{35,88,103-106} (Figure 2A).

To enter a host cell, the receptor-binding domain of the SARS-CoV-2 spike protein binds with ACE2 on the membrane\textsuperscript{87,107} (Figure 2B). Immediately, the virus either directly fuses with the cell membrane or the virus-ACE2 complex is endocytosed into the
host cell through a β₃-integrin-dependent process.108,109 Within endosomes, SARS-CoV-2 spike proteins, that were not cleaved by TMPRSS2 on the cell membrane, are cleaved by cathepsin L—the primary priming mechanism of pangolin sarbecornavirus spikes.103 Although the purpose of these spike cleavage events has not been fully appreciated, recent data suggest that spike cleavage is crucial for evading detection by interferon-induced transmembrane proteins (IFITM1, IFITM2, and IFITM3) so that the virus can properly fuse with the endosomal membrane.35,108

Although furin-mediated and TMPRSS2-mediated cleavage of SARS-CoV-2’s spike protein before ACE2-engagement is a canonical mechanism for SARS-CoV-2(A-D), mounting evidence suggests that SARS-CoV-2 might be able to infect lung cells that do not express ACE2.91 In addition, SARS-CoV-2 can exploit the Neuropilin-1 receptor,110,111 exploit the high-density lipoprotein scavenger type B receptor,112 and coat itself in soluble ACE2 and Vasopressin to gain access into cells, but these virus strategies remain to be further investigated. Cryo-electron microscopy has revealed that the spike protein on Omicron is more compact and stable than SARS-CoV-2(A-D) which is probably a conformational masking strategy to protect the interior receptor-binding domain from neutralizing antibodies.75,114 As Omicron has mutations surrounding its S2’ site, TMPRSS2-mediated cleavage of the spike protein is inefficient, and ACE2-mediated virus entry is markedly decreased in human cells.80,115 The trade-off is that Omicron is less dependent on TMPRSS2 processing of its spike protein before ACE2 receptor engagement, but now more dependent on cathepsin cleavage of its spike protein and clatherin-mediated endocytosis for infection.104 Therefore, Omicron seems to have reverted to its old self and is now more reliant on spike cleavage strategies employed by pangolin sarbecornaviruses.103

Once SARS-CoV-2 gains access to the cytoplasm of host cells, the release of its large RNA genome into the aqueous space triggers the initiation of a complex program of virus RNA translation.8 SARS-CoV-2 recruits host ribosomes to its 5’ end to translate two open reading frames (ORF), ORF1a and ribosomal-frameshift-dependent ORF1b, which constitute most of the genome.8 The products of translating these ORFs are two long amino acid sequences called pp1a and pp1ab.116 These two amino acid sequences are then proteolytically cleaved by the virus’ main protease (Mpro) on pp1a, which yields 16 mature non-structural proteins.8 Many of these effector proteins will go on to disrupt the splicing, translation, and protein trafficking of the host cell to prevent an antiviral Type I Interferon (IFN-I) response.117-119 Meanwhile, the RNA polymerase holoenzyme, that is formed by RdRp, nsp7, and nsp8, initiates RNA replication in double-membrane vesicles derived from the endoplasmic reticulum.89,116 The incoming newly synthesized negative-sense RNA will serve as a template for positive-sense genomic RNA and complementary positive-sense subgenomic RNAs.8,89,120 The translation of the subgenomic RNAs generates structural (e.g., Spike) and accessory proteins (e.g., ORF3a).120 These proteins along with nucleocapsid-enriched positive-sense genomic RNA are inserted into the ER-Golgi intermediate compartment to support the assembly and budding of an enveloped SARS-CoV-2 virus.8,89 Before lyosomal trafficking and exocytosis of the new virions occurs,121 the spike protein is precleaved by furin on the Golgi apparatus to prime the virus’ spike for ACE2-binding.85-87 Although most virions spread from infected cells through exocytosis, furin-mediated cleavage of the spike protein also allows SARS-CoV-2(A-D) to fuse infected cells with neighboring host cells to form a multinucleated cell, also known as syncytia.75,122 This was a common dissemination strategy used by SARS-CoV-2(A-D) in the lungs. However, since TMPRSS2 is required for syncytia formation sites,123 cell-cell fusion and virus spread by TMPRSS2-independent Omicron is severely impaired.115 Therefore, the loss of syncytia induction is another important factor that contributes to the attenuated fitness of Omicron.

2.3 The immune response to SARS-CoV-2 infection in the lungs

The detailed analyses of nasal lavage fluid, bronchoalveolar lavage fluid, and blood samples from innumerable COVID-19 patients has provided scientists and clinicians with a comprehensive model of the pathogenesis of COVID-19. Although our model of COVID-19 is constantly being refined, our fundamental understanding is that aberrant immune signaling in severe COVID-19 is precipitated by a delay in conventional antiviral responses.

At the beginning of infection, as SARS-CoV-2 spreads in the upper and lower respiratory tracts, innate immune sensors (PRRs) on a variety of host cells detect the pathogen-associated molecular patterns (PAMPs) of SARS-CoV-2, triggering robust cytokine production. For instance: the SARS-CoV-2 spike protein is sensed by Toll-Like Receptor 4 (TLR4),124,125 the envelope protein is sensed by TLR2,126 the nucleocapsid protein is sensed by the NLRP3 inflammasome,127 and viral RNA is sensed by retinoic acid-inducible gene-1 (RIG-I)128 and TLR7/8.129,130 Moreover, SARS-CoV-2 infection and syncytia of human lung pneumocytes and endothelial cells causes mitochondrial DNA release which stimulates the intracellular cGAS-STING pathway.131-133 The stimulation of these PRRs in
the lungs sets in motion a cascade of NF-κB-dependent signaling that leads to the potent and chronic release of pro-inflammatory cytokines, including TNFα, IL-1α, IL-1β, IL-6, and IL-8—all of which are consistently elevated in the blood of hospitalized COVID-19 patients\textsuperscript{134–136} and mice infected with mouse-adapted SARS-CoV-2 (MA10, MA30).\textsuperscript{137,138} Therefore, there is mounting evidence that the dramatic stimulation of PRRs, especially TLR2, NLRP3, and cGAS-STING, early in infection is sufficient to drive COVID-19 immunopathology in the lungs. The inhibition of these PRRs seems to prevent severe disease in SARS-CoV-2-infected laboratory mice and hamsters, indicating that these PRRs are not necessary to mount a successful immune response against SARS-CoV-2.

While cellular senescence in the lungs is a common consequence of SARS-CoV-2-induced hyperinflammation,\textsuperscript{139,140} preexisting cellular senescence is a risk factor for COVID-19. Besides the putative overexpression of SARS-CoV-2 entry factors in aged tissue,\textsuperscript{83,84} senescent cells have arrested antiviral responses.\textsuperscript{83,137,141} For instance, the overexpression of the lung senescence-associated Phospholipase A\textsubscript{2} Group 2D leads to dendritic cell impairment and poorer clinical outcomes in mice infected with mouse-adapted SARS-CoV (MA15) and SARS-CoV-2 (MA30).\textsuperscript{137,141} Senescent cells also have a tendency toward a more pro-inflammatory cytokine secretion state upon stimulation. Therefore, a positive-feedback loop of SARS-CoV-2 virus-induced cellular senescence in the lungs can further exacerbate hyperinflammation in aged individuals.\textsuperscript{142} Senolytic drugs are now being pursued as promising candidates for globally suppressing aberrant cytokine production in COVID-19.

It is generally believed that an early, antiviral type I IFN response promotes the clearance of the SARS-CoV-2 virus and attenuates SARS-CoV-2-induced hyperinflammation. However, similar to SARS-CoV and MERS-CoV, SARS-CoV-2 uses a variety of effector proteins to antagonize type I IFN production\textsuperscript{143–147} and suppress the induction of type I IFN-stimulated genes.\textsuperscript{148} Compared with orthologs from SARS-CoV and bat-derived SARS-like sarbecoronaviruses, SARS-CoV-2 effector proteins have become better at suppressing type I IFN induction; the subgenomic RNA expression and efficiency of these effector proteins is increasing with every new variant.\textsuperscript{145,149,150} Meanwhile, we can also be our own worst enemy—a common theme in severe COVID-19. For instance, Dr. Jean-Laurent Casanova and colleagues in 2016 provided us with the best demonstration of type I IFN induction being a double-edged sword during sarbecornavirus disease.\textsuperscript{158} In wildtype mice infected with hypervirulent SARS-CoV MA15, the administration of type I IFNs 6 hours postinfection prevents mortality, while the administration of type I IFNs 24 hours postinfection leads to widespread mortality.\textsuperscript{158} In contrast, mice deficient in the receptor for type I IFNs (IFNAR) were protected from MA15 mortality and had milder disease.\textsuperscript{158} Therefore, the timing of an all-or-none type I IFN response appears to dictate the severity of sarbecornavirus disease.\textsuperscript{158}

As previously described, senescent tissue carries a predilection toward dampened antiviral responses and hyperinflammation upon stimulation. Therefore, it is asserted that aged humans and animals mount a delayed type I IFN response against SARS-CoV-2 and that this subsequently sets the stage for an immune imbalance consisting of lymphopenia, monocytyosis, and neutrophilia.\textsuperscript{137,159–161} During late-stage severe COVID-19, proliferating neutrophils inundate the lungs and blood.\textsuperscript{162,163} The vicious release of neutrophil extracellular traps in blood vessels further exacerbates disease by causing rapid thrombosis.\textsuperscript{164} Meanwhile, inflammatory monocyte-derived macrophages (IMMs) flood the lungs and congregate in areas of high virus burden.\textsuperscript{137,165,166} Early data suggest that SARS-CoV-2-infected IMMs and TNFα-IFN-γ-stimulated IMMs undergo pyroptosis, a form of inflammatory cell death that is extremely deleterious for the lungs.\textsuperscript{130,167–169}

Ultimately, this complete dysregulation of the immune response allows SARS-CoV-2 to enter the blood—a critical moment because the circulating SARS-CoV-2 virus will then have direct access to a variety of organs including the brain. In addition, SARS-CoV-2-induced hyperinflammation in the lungs drives prominent damage in the lungs, including diffuse alveolar damage, thrombosis, and cellular senescence of epithelial and endothelial cells.\textsuperscript{137} All of these forms of cellular damage reduce the respiratory capacity of the lungs which leads to a dangerous hypoxic state in a variety of organs, especially the brain.\textsuperscript{170,171}

3 | SARS-CoV-2 PREFERENTIALLY TARGETS NON-NEURONAL CELLS

3.1 | SARS-CoV-2 infects the olfactory neuroepithelium

The olfactory neuroepithelium is an intimate site where apical, ciliated, glia-like sustentacular cells interact and communicate with basal bipolar olfactory sensory neurons. The exposed olfactory neuroepithelium that surrounds each ethmoid turbinate is lined with a thin mucus-rich cilia layer that is sheltered by a dense canopy of olfactory sensory neuron dendrites that are covered in a variety of odorant receptors.

Once airborne infectious SARS-CoV-2 virions are inhaled, the SARS-CoV-2 virus attempts to infect cells along the nasal respiratory epithelium and olfactory neuroepithelium.\textsuperscript{172,173} Sustentacular...
cells are especially vulnerable to SARS-CoV-2 infection because they display TMPRSS2 and ACE2 on their apical surface.\textsuperscript{101,174} Electron microscopy of the SARS-CoV-2-infected hamster neuroepithelium shows that sustentacular cells lose their cilia as SARS-CoV-2 fuses with the cell membrane.\textsuperscript{175} Once SARS-CoV-2\textsubscript{(A-D)} infects the columnar sustentacular cells, the virus gains access to a large portion of cytoplasm that extends into the basal lamina.\textsuperscript{173} Upon lysis of the sustentacular cells, virus progeny from the SARS-CoV-2-infected sustentacular cells will then bind to and infect neighboring cells that reside in the olfactory neuroepithelium.

SARS-CoV-2\textsubscript{(A-D)} infection of sustentacular cells is observed across species, including humans,\textsuperscript{172,173,175} humanized mice,\textsuperscript{176} wildtype mice,\textsuperscript{137,138} and hamsters.\textsuperscript{172,175} Interestingly, not all sustentacular cells are ACE2-expressing in mice—ACE2 is only localized to the apical surface of sustentacular cells residing in the NAD(P)H Quinone Dehydrogenase-rich dorsal ethmoid turbinate.\textsuperscript{101,174}

However, SARS-CoV-2\textsubscript{(A-D)} infection is observed throughout the ethmoid turbinates of wildtype mice, implying that SARS-CoV-2\textsubscript{(A-D)} can infect sustentacular cells through an ACE2-independent pathway.

Olfactory sensory neurons do not express TMPRSS2 or ACE2.\textsuperscript{101,174} Nevertheless, olfactory sensory neuron infection can occur on occasion. The frequency of olfactory sensory neuron infection seems to be age-dependent and species-dependent. SARS-CoV-2\textsubscript{(A-D)} fails to infect olfactory sensory neurons in adult humans,\textsuperscript{173} but SARS-CoV-2 readily infects the olfactory sensory neurons of young hamsters\textsuperscript{172,177} and adult deer mice.\textsuperscript{175} The overexpression of Neuropillin-1, a secondary entry receptor for SARS-CoV-2,\textsuperscript{110,111} in immature olfactory sensory neurons allows SARS-CoV-2 to travel along the olfactory nerve and invade the olfactory nerve layer of the olfactory bulb in young hamsters.\textsuperscript{172,175} Neuropillin-1 is expressed along the olfactory neuroepithelium of humans,\textsuperscript{110} but it remains to be seen whether SARS-CoV-2 utilizes the Neuropillin-1 receptor to infect human olfactory sensory neurons.

If the virus infection extends to the lower layer of the olfactory neuroepithelium, Bowman’s gland cells and horizontal basal cells might also become infected. Bowman’s gland cells, the main producers of mucin along the nasal epithelium,\textsuperscript{178} express high levels of TMPRSS2 but low levels of ACE2.\textsuperscript{101,174} SARS-CoV-2\textsubscript{(WA1)} and Omicron fail to infect the Bowman’s gland in Syrian hamsters, but SARS-CoV-2\textsubscript{(Delta)} gained the ability to infect Bowman’s gland cells.\textsuperscript{172} The selective advantage of SARS-CoV-2\textsubscript{(Delta)} in the Bowman’s gland could be due to its improved binding affinity for ACE2\textsuperscript{179} and its enhanced exploitation of TMPRSS2 for potentiating its spread among host cells.\textsuperscript{80,180}

Horizontal basal cells are the primary multipotent progenitors of the olfactory epithelium\textsuperscript{181} and give rise to sustentacular cells, Bowman’s gland cells, microvillous cells, and globose basal cells which are the direct progenitors of olfactory sensory neurons.\textsuperscript{182} Single-cell RNA-sequencing and immunofluorescence imaging of human biopsies confirmed that horizontal basal cells are TMPRSS2 and ACE2-expressing, but it remains unclear whether SARS-CoV-2\textsubscript{(A-D)} infects horizontal basal cells.\textsuperscript{101,174}

In 2020, a medical group in Germany released their initial evaluations of postmortem olfactory tissue from COVID-19 patients and showed that, in some instances, SARS-CoV-2 RNA can be detected in the human olfactory bulb.\textsuperscript{183} They concluded that SARS-CoV-2 is neurotropic and can travel along the olfactory nerve to infect the olfactory bulb,\textsuperscript{183} as observed in HCoV-OC43\textsuperscript{184} and MHV-JHM\textsuperscript{185} betacoronavirus infections. Upon further exploration, SARS-CoV-2 RNA could also be detected in the olfactory bulbs of SARS-CoV-2-infected African Green monkeys,\textsuperscript{37} Rhesus monkeys,\textsuperscript{186} and Syrian hamsters.\textsuperscript{175} These reports conflicted with data showing that the mammalian olfactory bulb parenchyma was devoid of ACE2 and TMPRSS2.\textsuperscript{101,174} However, the discovery of Neuropillin-1 as a secondary receptor for SARS-CoV-2 renewed theories of neuronal anterograde transport of SARS-CoV-2 to the olfactory bulb.\textsuperscript{110,111} To obtain definitive proof of SARS-CoV-2 infection in the human olfactory bulb parenchyma, Khan and coworkers performed spatial transcriptomics on human olfactory bulbs to holistically detect SARS-CoV-2 RNA and pinpoint its location in the tissue.\textsuperscript{173} On the contrary, they discovered that SARS-CoV-2 was only localized to blood vessels and the leptomeninges, and virus was not detected in the parenchyma.\textsuperscript{173} The detection of SARS-CoV-2 RNA in the human olfactory bulb is most likely due to the virus remaining attached to endothelium.\textsuperscript{146} In sum, SARS-CoV-2 efficiently infects the olfactory neuroepithelium but not the olfactory bulb of the olfactory system.

### 3.2 SARS-CoV-2 Infects the Cerebrovasculature and Choroid Plexus

Neurological complications and cognitive impairments arising during severe COVID-19 have led to a great deal of speculation regarding the neurotropism and neuroinvasiveness of SARS-CoV-2 in the brain. When SARS-CoV-2 viremia occurs in severe COVID-19, the circulating virus has access to a variety of organs including the brain. SARS-CoV-2\textsubscript{(A-D)} is frequently detected in postmortem brains of older COVID-19 patients.\textsuperscript{170,183,187} As in humans, low levels of SARS-CoV-2\textsubscript{(A-D)} can be detected on occasion in the brains of non-human primates\textsuperscript{37,186} and ferrets.\textsuperscript{40,43} SARS-CoV-2\textsubscript{(A-D)} RNA is plentiful in the brains of young hamsters\textsuperscript{36,53,175} but has yet to be detected in the brains of infected standard laboratory adult mice,\textsuperscript{137,138,188} and adult hamsters.\textsuperscript{53} Further research is needed to assess whether SARS-CoV-2 RNA can be detected in the brains of aged rodents to recapitulate human disease.

Despite the frequency of viral RNA detected in the human brain, SARS-CoV-2 is only localized to blood vessels and fails to invade the brain parenchyma, according to immunohistochemistry and spatial transcriptomics of human samples.\textsuperscript{146,170,173,183,189,190} These observations are consistent with the localization of ACE2 and Neuropillin-1 along blood vessels of mouse and human brains, but not the parenchyma.\textsuperscript{101,146,174} implying that the requirements needed for efficient SARS-CoV-2 infection only exist at this site. However, pericytes are the vascular cell that highly express ACE2,\textsuperscript{101,146,191} and they remain hidden from SARS-CoV-2 behind the tight blood-brain barrier.
Endothelial cells do not express ACE2 and have a low expression of Neuropilin-1. TMPRSS2 is also not detected along the cerebrovasculature. Undeterred by these contradictions, Wenzel and coworkers brought forth direct evidence that SARS-CoV-2 infects brain endothelial cells and facilitates their death. The main protease (Mpro) of SARS-CoV-2 cleaves endothelial cells’ nuclear factor (NF)-κB essential modulator (NEMO), an essential protein for cell survival in an inflammatory environment. While the inhibition of NEMO is part of a concerted effort by multiple SARS-CoV-2 proteins to prevent RIG-I-mediated NF-κB induction, the loss of NF-κB has the unintended consequence of sensitizing cells for TNFα-induced, RIPK1-mediated cell death. In the inflamed intestines, this loss of NF-κB and subsequent cell apoptosis compromises barrier integrity, and the same holds true with the blood-brain barrier. As a result, the SARS-CoV-2-infected blood vessels collapse, forming empty basement membranes, known as string vessels, throughout the brains of SARS-CoV-2-infected hamsters and COVID-19 patients. String vessel formation caused by NEMO inhibition instigates cortical astrogliosis. Recently, Yang and coworkers showed that inflammatory astrocytes account for approximately 80% of the differentially expressed genes in the frontal cortex of COVID-19 patients — SARS-CoV-2-induced string vessel formation is a likely contributor to this phenotype. To perturb SARS-CoV-2-induced string vessels and possibly suppress thrombotic events and inflammatory astrocytes in the COVID-19 brain, Pfizer’s FDA-approved Mpro inhibitor, Paxlovid, is a promising preventative.

To identify the vulnerable cell types beyond the brain endothelium that SARS-CoV-2 has the capacity to infect, multiple laboratories have set out to create human cortical organoid models of SARS-CoV-2 infection. Despite their efforts, each laboratory’s conclusion contradicts the next. However, laboratories utilizing SARS-CoV-2-infected brain organoids have reached agreement on the identity of one vulnerable cell population in the brain: choroid plexus epithelial cells. This comes as no surprise because SARS-CoV-2 infects and replicates in a variety of epithelium. The choroid plexus, a cerebrospinal fluid-secreting tissue, could be an important site of SARS-CoV-2 dissemination in the central nervous system during severe COVID-19. SARS-CoV-2 is occasionally observed within the capillaries of the human choroid plexus. Human choroid plexus organoid models show that SARS-CoV-2 infects the choroid plexus epithelium, which leads to syncytia formation, the loss of homeostatic barrier integrity and ion transport, and the upregulation of signaling pathways involved in cell death and inflammatory cytokine production. A subset of apolipoprotein-producing mature choroid plexus cells express high levels of ACE2 and TMPRSS2 and are especially vulnerable to SARS-CoV-2 infection. These apolipoprotein-producing choroid plexus cells increase their expression of ACE2, APOJ (Clusterin), and APOE as the virus infects and damages the organoid epithelium. Neurons and glia expressing APOE4 are especially vulnerable to SARS-CoV-2 in vitro, and this might also be the case with choroid plexus epithelial cells. Although it is unclear how APOE is aiding in virus entry, a human-SARS-CoV-2 protein interactome developed from HEK-293T cells illuminated lipoprotein metabolic machinery as the primary target of SARS-CoV-2’s spike protein. Moreover, the presence of high-density lipoproteins and the high-density lipoprotein scavenger receptor type B enhances SARS-CoV-2 infection in lung cells. Flaviviruses, such as the Hepatitis C Virus, form structures with lipoproteins called lipoviroparticles that aid in virus spread and entry into host cells. The possibility that SARS-CoV-2 has gained the ability to use lipoproteins produced by the choroid plexus as Trojan horse lipoviroparticles should be taken seriously.

There have been some unusual cases of SARS-CoV-2 being detected in the cerebrospinal fluid of patients experiencing meningitis and encephalitis. Although organoid models do not exactly phenocopy human disease, the studies discussed in this subsection of the review indicate that choroid plexus epithelial cells might release SARS-CoV-2 into the cerebrospinal fluid through rampant virus shedding and syncytia formation. Future research is needed to characterize the unusual role lipoproteins may have in facilitating SARS-CoV-2 spread along the blood vessels and choroid plexus of the brain.

4 | THE HOST’S NEUROIMMUNE RESPONSE IN THE OLFACTORY SYSTEM DURING SARS-COV-2 INFECTION

4.1 | SARS-CoV-2 infection downregulates homeostatic olfaction-related gene expression

SARS-CoV-2-induced anosmia has renewed interest in the neuroscience community to further elucidate the complex processes that are required for olfaction. Gradually, we are beginning to understand that the olfactory system has dynamic neuroimmune underpinnings, and SARS-CoV-2 has exposed its ON-OFF switch. When SARS-CoV-2 begins infecting cells along the olfactory neuroepithelium of humans and hamsters, there is a widespread downregulation in the expression of thousands of homeostatic olfaction-related genes. For instance, even in areas with negligible virus, the gene expression of adenylyl cyclase 3, an important enzyme for odorant receptor signal transduction and specialization, is shut off during SARS-CoV-2 infection. Genes encoding olfactory sensory neuron receptors are also downregulated in humans and hamsters during SARS-CoV-2 infection (Figure 3). Olfactory sensory neuron receptor expression returns to normal in anosmic patients postinfection, which indicates that receptor loss is probably not the culprit in SARS-CoV-2-induced anosmia. Instead, sustentacular cells, the primary targets of SARS-CoV-2 in the olfactory neuroepithelium, have dysregulated gene expression in anosmic patients.
After months recovering from SARS-CoV-2 infection, human sustentacular cells overwhelmingly express genes related to antigen presentation and interferon signaling. With the discovery that SARS-CoV-2 can persist in the human olfactory mucosa for months postinfection, it is possible that sustentacular cells express this antiviral phenotype in response to an underlying, chronic infection.

4.2 Immune cells migrate to the SARS-CoV-2-infected olfactory neuroepithelium

SARS-CoV-2(A-D) infection of the olfactory neuroepithelium causes an influx of a variety of immune cells into the ethmoid turbinates. In the olfactory neuroepithelium and lamina propria, resident macrophages survey the environment to recognize and neutralize pathogens. In the incipient stages of SARS-CoV-2(A-D) infection, levels of macrophage chemotaxants, including CCL5 and CXCL10, become elevated in the ethmoid turbinates of hamsters (Figure 3). As the infection of the ethmoid turbinates progresses, an increasing number of Iba1+ macrophages infiltrate the neuroepithelium and swarm the sites of SARS-CoV-2 infection (Figure 3). This robust macrophage response in the olfactory neuroepithelium is consistent with the high levels of Macrophage Inflammatory Protein-1 Alpha (MIP-1α) detected in the ethmoid turbinates of SARS-CoV-2(A-D)-infected hamsters. The increased presence of granulocytes in the human nasal cavity and myeloperoxidase-containing cells in the hamster ethmoid turbinates has also been detected during acute infection (Figure 4). Elevated CD4+ cell counts in the nasopharyngeal swabs of COVID-19 patients and increased gene expression of CD3, CD4, and CD86 in the ethmoid turbinates of SARS-CoV-2-infected hamsters suggests that activated CD4+ T cells flood the ethmoid turbinates to aid in the clearance of SARS-CoV-2. Mass cytometry of human nasopharyngeal swabs and RNA-sequencing of the hamster ethmoid turbinates has confirmed that a large population of CD8+ T cells and Natural killer (NK) cells inundate the neuroepithelium during peak infection. Cytotoxic factors, such as Perforin and Granzymes, are highly expressed in the SARS-CoV-2-infected hamster olfactory neuroepithelium (Figure 3), suggesting that CD8+ T cells and NK cells are potentially involved in the eradication of SARS-CoV-2-infected cells (Figure 4). Nasal brushing of former COVID-19 patients shows that a large IFN-γ+ CD8+ T cell population can remain in the nasal mucosa at least 2-16 months postinfection.

Overall, due to insufficient studies characterizing the identities of immune cells in the ethmoid turbinates, there is a major gap in our knowledge of how the immune response to nasal SARS-CoV-2 infection is orchestrated and whether these immune activities are detrimental to the surrounding nervous system milieu. Filling this gap in knowledge will ultimately pave the way for designing next-generation intranasal vaccines against SARS-CoV-2 to prevent its spread and the anosmia that it causes.
4.3 | Sloughing of the olfactory neuroepithelium during SARS-CoV-2 infection

As SARS-CoV-2(A-D) continues to infect host cells along the olfactory neuroepithelium of humans and rodents, the structure of the neuroepithelium is destabilized which facilitates the sloughing (i.e., desquamation) of infected and non-infected cells into the nasal lumen.\textsuperscript{172,175,177,215} During SARS-CoV-2\textsubscript{(A-D)} infection, olfactory sensory neurons and sustentacular cells are released into the luminal space.\textsuperscript{172,175} Similar to the process of anoikis in the intestinal epithelium, apoptosis only occurs after the detachment of the cell from the olfactory neuroepithelium.\textsuperscript{175,216} This sloughing of the olfactory neuroepithelium occurs through an unknown mechanism that appears to be neutrophil-mediated.\textsuperscript{216} Treatment with a Cathepsin C inhibitor reduced the amount of cells expelled into the nasal lumen following SARS-CoV-2\textsubscript{(Beta)} intranasal inoculation of Syrian golden hamsters.\textsuperscript{216} It seems likely that the sloughing of the olfactory neuroepithelium is a protective mechanism to clear pathogens from the ethmoid turbinates. However, Cathepsin C inhibition and suppression of sloughing limited SARS-CoV-2 spread along the olfactory neuroepithelium.\textsuperscript{216} Therefore, the sloughing of infected cells could instead aid in spreading infectious virions to non-infected sites of respiratory epithelium and olfactory epithelium. In theory, SARS-CoV-2-infected sloughed cells could also travel from the nasal cavity to the larynx via “post-nasal drip,” providing SARS-CoV-2 access to highly vulnerable, ACE2-expressing acini and duct cells in the salivary gland mucosa.\textsuperscript{218}

We have yet to fully understand the extent of the sloughing in the olfactory neuroepithelium during SARS-CoV-2(A-D) infection and...
how much of this phenomenon contributes to SARS-CoV-2-induced anosmia. Olfactory sensory neurons are one of the cell types that are sloughed into the lumen so the loss of these neurons may alter normal olfaction. A study with SARS-CoV-2(WA1)-infected Syrian hamsters asserts that the sloughing of the olfactory neuroepithelium thickness along the ethmoid turbinates negatively correlates with olfactory function, according to a buried food test. The intensity of sloughing along the olfactory neuroepithelium could be a measure to predict the duration of prolonged anosmia following infection.

If the vast majority of cells along the olfactory neuroepithelium are sloughed off, horizontal basal cells will need more time to multiply and give rise to an intact, multilayered olfactory neuroepithelium that supports normal olfaction. It is unknown whether horizontal basal cells are also sloughed into the lumen during infection. If the death or sloughing of horizontal basal cell does occur as observed in dichlorobenzonitrile poisoning, this would further delay and possibly prevent the regeneration of the olfactory neuroepithelium.

The expulsion of the olfactory neuroepithelium has been observed in healthy and cyclophosphamide-immunosuppressed hamsters infected with SARS-CoV, demonstrating that this is a phenotype that is conserved across sarbecovirus infections. In contrast, sloughing of the olfactory neuroepithelium is not observed during Omicron infection in rodents which is most likely due to the lack of efficient Omicron replication in the ethmoid turbinates. Although the frequency of anosmia among COVID-19 patients infected with Omicron is slightly reduced, anosmia is still a common symptom for patients infected with Omicron indicating that sloughing cannot be the only factor that contributes to SARS-CoV-2-induced anosmia.

4.4 The repair of the olfactory neuroepithelium during SARS-CoV-2 infection

When the olfactory neuroepithelium is damaged by a pathogen, toxin, or physical trauma, horizontal basal cells are needed to regenerate the many layers of the epithelium. Within 18 hours postinjury in the murine olfactory neuroepithelium, horizontal basal cells downregulate their p63-controlled dormancy state and become activated, proliferative stem cells. Within 4 days postinfection, a large population of active, Ki67-cycling horizontal basal cells is observed in the olfactory neuroepithelium of SARS-CoV-2(A-D)-infected hamsters. Moreover, the expression of horizontal basal cell markers is upregulated in the ethmoid turbinates of hamsters during early acute SARS-CoV-2 infection. It is unknown how early horizontal basal cells start self-renewing during SARS-CoV-2 infection in humans. Regeneration following SARS-CoV-2 infection could vary by species. According to tongue biopsies of COVID-19 patients, the stem cell layer of the fungiform papillae taste cells can have reduced cell mitosis for at least 6-weeks postinfection, contributing to the prolonged loss of taste that COVID-19 patients experience. Therefore, it is possible that horizontal basal cell proliferation is hindered for weeks post-SARS-CoV-2 infection in humans.

Chronic rhinosinusitis experiments suggest that the regenerative capacity of horizontal basal cells is silenced by chronic inflammation. NF-κB-activation of horizontal basal cells causes these cells to release pro-inflammatory factors such as CXCL10 to support the local proliferation of macrophages and T cells. Pro-inflammatory factors such as CXCL10 and immune cells including granulocytes and resident memory T cells remain elevated in the human nasal cavity months after SARS-CoV-2 infection. Therefore, it is quite possible that the proliferative capacity of horizontal basal cells is suppressed by chronic inflammation postinfection thus abrogating the regeneration of the olfactory neuroepithelium. This phenomenon could be a major cause of prolonged SARS-CoV-2-induced anosmia.

SARS-CoV-2 infection of sustentacular cells and their subsequent desquamation from the neuroepithelium could have a negative impact on the horizontal basal cells’ capacity to regenerate olfactory sensory neurons. The olfactory neuroepithelium has an olfactory sensory neuron-independent Jagged1-notch1 fail-safe mechanism in place to facilitate the replenishment of sustentacular cells following injury or ablation. However, to our knowledge, all identified mechanisms for the replenishment of olfactory sensory neurons following injury are sustentacular cell dependent. In theory, as the somas and dendrites of olfactory sensory neurons are sloughed off following damage to the neuroepithelium, extracellular ATP levels rise in the nasal lumen. Sustentacular cells detect this increase in extracellular ATP via their purinergic receptors. According to bulk RNA-sequencing, active SARS-CoV-2 infection of the hamster olfactory neuroepithelium promotes the overexpression of P2RY1, P2RY2, P2RY6, P2RY12, P2RY13, and P2RY14. Once these purinergic receptors are activated on sustentacular cells, the cell membrane becomes hyperpolarized by the release of calcium from intracellular reserves and the influx of potassium. As a result, neighboring sustentacular cells synchronize their hyperpolarization oscillations through gap junctions to generate the secretion of neurotrophic neuropeptide Y.

Activation of neuropeptide Y receptors on horizontal basal cells evokes p44/42 ERK-mediated differentiation of horizontal basal cells into immature olfactory sensory neurons. The gene encoding neuropeptide Y is one of the most downregulated genes in the nasal neuroepithelium of SARS-CoV-2-infected hamsters. Therefore, as SARS-CoV-2 infection causes rampant sloughing of sustentacular cells, the restoration of the neuropeptide Y-secreting sustentacular cell population might be required before horizontal basal cells can start replenishing the olfactory sensory neuron population during recovery. Hinderance to any of these regenerative processes might explain the low olfactory sensory neuron to sustentacular cell ratio in the olfactory epithelium of anosmic COVID-19 patients postinfection.
SARS-CoV-2-induced anosmia across species

Approximately 70% of SARS-CoV-2-infected, PCR-positive individuals self-report anosmia.\textsuperscript{239} Quantitative olfactometry on former COVID-19 patients determined that olfactory dysfunction persists in approximately 60% of individuals for at least 6 months postinfection.\textsuperscript{240} Many research groups claim that SARS-CoV-2-induced anosmia can be recapitulated in humanized mice, hamsters, and zebrafish, but these assertions should be taken with caution. For instance, zebrafish inoculated with the SARS-CoV-2 Spike receptor-binding domain display “impaired olfaction,” according to a food odor choice test.\textsuperscript{241} However, this behavior impairment is likely the result of global neurological deficits. There is mounting evidence that the SARS-CoV-2 Spike protein’s receptor-binding domain has sequence homology with neurotoxins\textsuperscript{242} and causes rampant neurotoxicity in aquatic life.\textsuperscript{243}

Meanwhile, the buried food test has been relied upon to study SARS-CoV-2-induced anosmia with rodent models. SARS-CoV-2(A-D)-infected humanized mice and hamsters have exhibited “decreased olfactory function” by finding buried food less often during infection.\textsuperscript{175,176,219} However, the buried food test has major caveats and there are problems with how the behavior test was performed in these studies. To aid the field in developing an animal model for SARS-CoV-2-induced anosmia, here are some guidelines that should be followed to increase the validity and reliability of buried food tests for assessing SARS-CoV-2-induced anosmia.

First, as rodents perform the buried food test daily for 3 weeks, their latency to find the buried food drastically decreases from a latency of ~200 to ~50 s.\textsuperscript{244} This is evidence that olfaction sensitivity or memory recall for a specific odor stimulus increases over time. To account for this confounding variable, experimenters should repeat the buried food test daily in naïve mice until their latency to find the buried food plateaus.\textsuperscript{244} Once the latency to find the buried food has plateaued during the training phase, SARS-CoV-2 inoculation can occur to observe changes in food finding.

Second, decreased food retrieval during SARS-CoV-2 infection can be due to sickness behavior. The lack of appetite or activity during SARS-CoV-2 infection could alter the food finding abilities of rodents. To partially account for this confounding variable, the movement of the mice in the buried food test arena should be quantified. Mock-infected mice can be treated with pharmacological agents (e.g., methimazole) to transiently impair olfaction without sickness behavior, thus serving as appropriate positive controls in the experiment.\textsuperscript{244}

Overall, due to the intensive labor required for training animals for reliable olfaction-related behavior tests in a biosafety level 3 laboratory, it is recommended that the field shifts toward quantitative olfactometry. Plethysmography of odor-evoked sniffing would be a more reliable method for measuring olfaction in SARS-CoV-2-infected rodents even if they are presenting with sickness behavior and reduced appetite.

5 | THE HOST’S NEUROIMMUNE RESPONSE IN THE BRAIN DURING SARS-COV-2 INFECTION

5.1 | SARS-CoV-2 infection promotes antiviral activity along the cerebrovasculature and choroid plexus

Recent studies utilizing single-nucleus RNA-sequencing and spatial transcriptomics suggest that robust interferon-related signaling is occurring along the blood-brain barrier (BBB) of COVID-19 patients.\textsuperscript{189,245} IFITM1 and IFITM2 expression is upregulated along the blood vessels of the frontal cortex,\textsuperscript{245} while IFITM3 is highly upregulated in cortical astrocytes and in all types of cells residing in the choroid plexus of COVID-19 patient brains.\textsuperscript{189} Although IFITM activity constrains the successful entry of pseudotyped SARS-CoV\textsuperscript{35,246} and MERS-CoV\textsuperscript{247} in vitro, bat-derived SARS-like viruses and SARS-CoV-2 can partially evade IFITM detection in the endosome through TMPRSS2-dependent processes.\textsuperscript{35,246-250} Upon further exploration, it was discovered that the accumulation of IFITM3 on the plasma membrane promotes, rather than restricts, SARS-CoV-2 infection of host cells in vitro.\textsuperscript{250} Hence, enhanced IFITM activity along the barriers of the brain might decrease the frequency of SARS-CoV-2-endosome fusion but increase the frequency of SARS-CoV-2-plasma membrane fusion on the blood-brain barrier. An overexpression of genes related to antigen processing and major histocompatibility complex class I (MHC I) antigen presentation is also observed in the neurovascular unit during severe COVID-19,\textsuperscript{245} which is most likely the result of both IFN-γ stimulation\textsuperscript{251} and SARS-CoV-2 directly infecting endothelial cells.\textsuperscript{146}

As the choroid plexus is a vulnerable location for SARS-CoV-2 infection, it should come as no surprise that monocytes/macrophages account for a significant fraction of total cells in the choroid plexus of COVID-19 patients compared with control patients.\textsuperscript{252} It is unclear whether this large monocyte/macrophage population in the choroid plexus is further infiltrating the surrounding periventricular parenchyma during COVID-19. Markers of macrophage activation, complement signaling, and oxidative stress are upregulated in the choroid plexus.\textsuperscript{189} –all of which are common immune processes observed in SARS-CoV-2-infected tissues. Meanwhile, the cellular fraction of mesenchymal cells is also highly elevated in the choroid plexus of COVID-19 patients.\textsuperscript{189,252} Throughout life, choroid plexus mesenchymal cells express colony-stimulating factor 1 (CSF-1), which is an important signal for macrophage/microglia survival in the brain.\textsuperscript{253} Further research is needed to determine whether CSF-1-producing mesenchymal cells are crucial for the preservation of CSF1R\textsuperscript{+} macrophage populations and macrophage-mediated antiviral activity in the choroid plexus.
5.2 | Microglia nodules form in the brain parenchyma during severe COVID-19

To date, there is a lack of evidence that SARS-CoV-2 (A-D) invades the brain parenchyma. Instead, the nidus of SARS-CoV-2 (A-D) infection in the CNS is localized to the cerebrovasculature. Large populations of perivascular macrophages and T cells are frequently observed congregating around blood vessels to presumably prevent further infection in the brains of COVID-19 patients.170,187,189,190,254

However, the most intense pro-inflammatory activity in the COVID-19 brain occurs in the parenchyma. Microgliosis, perivascular macrophage accumulation, and microglia aggregates, known as nodules, are frequently observed in the brain parenchyma of patients who succumb to COVID-19170,187,189 (Figure 4). In line with these findings, microglia in the frontal cortex exhibit a variety of differentially expressed genes, including those associated with antigen presentation,189 iron storage,189 and NK cell-mediated cytotoxicity.252

The formation of microglia nodules in the COVID-19 brain has received growing attention because nodules are associated with axon damage in viral encephalitis,255–257 multiple sclerosis,258–260 Rasmussen encephalitis,261,262 and aging white matter.263 Despite the close proximity of the olfactory bulb to the SARS-CoV-2-infected and inflamed olfactory neuroepithelium, few microglia nodules are found there.190 Instead, caudal locations of the human COVID-19 brain, especially the medulla andpons, are burdened with microglia nodules.170,190 Whether it is the direct result of SARS-CoV-2 killing endothelial cells or the indirect result of chronic systemic inflammation, the BBB loses its integrity and neurovascular leakage of fibrinogen, a liver-derived protein, is observed in the brain parenchyma of COVID-19 patients.190,254 The buildup of fibrinogen in the parenchyma may contribute to the clustering of activated perivascular macrophages and microglia in the tissue264 (Figure 4). In addition, the increased BBB permeability allows CD8+ T cells to infiltrate the brain parenchyma.190 If the pathogenesis of COVID-19 microglia nodules mimics other encephalitides featuring nodules, the infiltrating CD8+ T cells will migrate to pre-form microglia nodules.265 Within the microglia nodules of the medulla, a heterogenous population of CD8+ T cells display phenotypic markers of activated effector T cells, cytotoxic T cells, proliferating T cells, and exhausted T cells.190 It is hypothesized that intimate and extensive crosstalk between microglia and infiltrating CD8+ T cells is occurring at these sites. For instance, the high density of MHC II+ microglia and PD-L1+ microglia within these nodules suggests that microglia are presenting antigens while actively regulating the responses of the CD8+ T cells.190 In chronic active multiple sclerosis lesions, HLA-DR+ microglia nodules are co-localized with amyloid-precursor protein (APP) deposits, which is indicative of axonal injury following demyelination.260

Surprisingly, abnormal APP deposits were observed in the brains of COVID-19 patients with high microglia nodule burden.190 To date, no histopathological features of demyelination have been observed in the COVID-19 brain. Yet, if microglia-mediated demyelination or axonal injury is happening in the COVID-19 brain, the observed neurovascular leakage of fibrinogen in COVID-19 will prevent any remyelination efforts. Fibrinogen inhibits oligodendrocyte progenitor cell (OPC) differentiation and suppresses remyelination by activating the bone morphogenetic protein (BMP) signaling pathway.265

Further investigation will be needed to illuminate the mechanistic role of microglia nodules in the COVID-19 brain. From the limited number of postmortem examinations of brains from COVID-19 patients, it is difficult to ascertain if microglia nodules are localized to only the white matter, as observed in autoimmune diseases,258,262 or if microglia take on a diffuse patterning, as is often observed in viral encephalitis.257 In addition, should microglia nodules prove to have a pathogenic role in the COVID-19 brain, Triggering Receptor Expressed on Myeloid Cells 2 (TREM2)-antagonizing therapies could be a promising treatment because microglia nodule formation requires TREM2-mediated phagocytosis.263,266 Most of all, it is crucial to uncover which antigens are being presented by microglia to CD8+ T-cells in the nodules; self-antigens would be indicative of an attack-on-self, whereas SARS-CoV-2 antigens would suggest an overprotective immune response.

5.3 | Evidence of a complex innate and adaptive immune response in the central nervous system of neuro-PASC patients

Some survivors of COVID-19 experience persistent neuro-PASC (postacute sequelae of SARS) for many months postinfection. Although the immune response in the CSF does not mirror the brain, the extraction of CSF from these COVID-19 survivors provides us with insights into the immune processes occurring in the CSF-secreting areas of the brain (choroid plexus and interstitial tissue) following infection. Our first set of clues came from Heming and co-workers: similar to the widespread T cell exhaustion detected in the blood of COVID-19 patients,267,268 a neuro-PASC patient has an expanded population of “exhausted” CD4+ T cells in their CSF compared to control patients with virus encephalitis.269 Exhausted T cells are frequently associated with inadequate clearance of chronic infections, but all neuro-PASC CSF samples in the study were negative for SARS-CoV-2.269 The CSF of neuro-PASC patients also exhibit greater proportions of border-associated macrophages, microglia, and Clec10a+ granulocytes than control patients with virus encephalitis.269

IL-12 plays a pivotal and non-redundant role in generating IFN-γ-producing NK cells and priming CD4+ and CD8+ T cells for adaptive immune responses in the brain, especially during Toxoplasma gondii infection.270 CSF levels of IL-12 are elevated in neuro-PASC patients, while the IL-12R receptor is highly expressed in the surrounding CD4+ and CD8+ T cell CSF populations of neuro-PASC patients.271,272 Despite a robust NK cell activation state in the blood of COVID-19 patients during infection,273 NK cells from the CSF of patients with neuro-PASC have enhanced expression of genes associated with NK cell-mediated cytotoxicity, antigen presentation, and chemoattraction, compared to plasma NK cells from the same neuro-PASC patients.271 Since SARS-CoV-2 infection of host cells
can yield cellular senescence, it is plausible that these NK cells are eliminating senescent cells in the brain—a special NK cell activity that occurs during normal brain aging. 274 It remains unclear which cells are secreting IL-12 in the CNS and what the motive might be for IL-12 polarizing NK, CD4+, and CD8+ T cell responses in the CNS during neuro-PASC.

Meanwhile, there is mounting evidence of a highly active adaptive immune response in the CSF of neuro-PASC patients. Consistent with the high number of infiltrating B cells in the parenchyma of patients with fatal COVID-19, 190 an increased frequency of B cells also exists in the CSF of neuro-PASC patients. 271 Some IgG antibodies produced by these B cells in neuro-PASC patients exhibit anti-neural reactivity, so more studies are needed to determine whether neuro-PASC has autoimmune underpinnings.

6 | CONCLUSION

The prevailing theory is that an overactive pro-inflammatory immune response, during a hypoxic state, drives neuroinflammation and damage in the CNS of hospitalized COVID-19 patients. The blood of hospitalized COVID-19 patients is filled with pro-inflammatory cytokines, and this dysregulated immune profile persists for months postinfection. 275 Building on this premise, high neurofilament light-chain levels in the serum and CSF of hospitalized COVID-19 patients are positively correlated with disease severity, suggesting that axonal injury occurs in tandem with global hyperinflammation during COVID-19. 276–278 As a result, 6 months following hospital discharge, hospitalized COVID-19 patients have an excess burden of ~60 per 1,000 persons with chronic fatigue and an excess burden of ~40 per 1,000 persons with memory problems, compared to PCR-negative hospitalized patients. 277 The inability to recover from these neuro-PASC complications for many previously hospitalized patients suggests that COVID-19-associated hyperinflammation and hypoxia have sowed the seeds for an underlying disease. Based on the current findings outlined in this review and the increased frequency of neuro-PASC symptoms occurring in older individuals, 280 it is plausible that SARS-CoV-2 can exacerbate or trigger Alzheimer’s Disease (AD) in older patients.

COVID-19 and AD share common traits. For instance, the orbitofrontal cortex and the entorhinal cortex within the parahippocampal gyrus have been identified through autopsies and positron emission tomography scanning of brains as two of the initial sites of early AD, consisting of phosphorylated tau tangles and beta-amyloid (Aβ) aggregates. 281–283 The presence of tau and Aβ in the orbitofrontal cortex and parahippocampal gyrus positively correlate with gray matter loss in those areas in early AD. 284 Similarly, magnetic resonance imaging of brains from 785 UK Biobank participants revealed that, following SARS-CoV-2 infection, COVID-19 patients have reduced gray matter thickness in their orbitofrontal cortex and parahippocampal gyrus. 285 Another common trait between COVID-19 and AD is the APOE4 allele, which may support SARS-CoV-2 tropism. Two copies of the APOE4 allele raise an individual’s risk of developing AD and severe COVID-19. 287 Most of all, anosmia, a common symptom of COVID-19, enhances the risk of a person carrying the APOE4 allele to develop AD. 288

Another major takeaway from this review is that bat-derived sarbecoroviruses, which have a high frequency of crossing the species barrier and an uncanny ability to adapt to new hosts without sacrificing virulence factors, are a constant threat to the global human population. 18,289 We now know that bat-derived sarbecoroviruses are already poised for human emergence again. 290 In addition, SARS-CoV-2 has established a new natural reservoir for sarbecoroviruses in a variety of species. Because SARS-CoV-2 is neurotropic in the olfactory systems of wild rodent species, there is now the worry that sarbecoroviruses will become increasingly acquainted with the mammalian nervous system. Therefore, with no pan-sarbecorovirus vaccine in sight, there is a considerable need to further investigate how SARS-CoV-2 damages the peripheral olfactory system and central nervous system to prepare for future sarbecorovirus pandemics and the potential neuro-PASC epidemics that follow.

AUTHOR CONTRIBUTIONS
All authors contributed to the writing, review, and editing of the manuscript.

ACKNOWLEDGEMENTS
We apologize to authors whose work could not be referenced in this review due to space limitations. Graphical illustrations were made using BioRender (https://biorender.com/). This work was supported by grants from the Manning Family Foundation (awarded to W.A.P.), Ivy Foundation (awarded to W.A.P.), Henske Family (awarded to W.A.P.), NIH R01 AI124214 (awarded to W.A.P.), NIH R01 NS106383 (awarded to J.R.L.), The Alzheimer’s Association AARG-18-566113 (awarded to J.R.L.), and The Owens Family Foundation (awarded to J.R.L.). N.R.N. was supported by the Global Biothreats Training Grant (NIH T32 AI055432-19).

CONFLICT OF INTEREST
The authors declare no competing financial interests.

DATA AVAILABILITY STATEMENT
Not applicable.

ORCID
John R. Lukens https://orcid.org/0000-0002-6795-0866
William A. Petri Jr https://orcid.org/0000-0002-7268-1218

REFERENCES
1. Zhou P, Yang XL, Wang XG, et al. A pneumonia outbreak associated with a new coronavirus of probable bat origin. Nature. 2020;579(7798):270–273. doi:10.1038/s41586-020-2012-7
2. Merad M, Blish CA, Sallusto F, Iwasaki A. The immunology and immunopathology of COVID-19. Science. 2022;375(6585):1122-1127.
3. Whitaker M, Elliott J, Bodinier B, et al. Variant-specific symptoms of COVID-19 among 1,542,510 people in England.
95. Weis W, Giffen V, Tostanoski LH, et al. Reduced pathogenicity of the SARS-CoV-2 omicron variant in hamsters. Med 2022;3(4):262-268.e4. doi:10.1016/j.med.j.2022.03.004

96. Shuai H, Chan JFW, Hu B, et al. Attenuated replication and pathogenicity of SARS-CoV-2 in newborn mice. Cell Rep 2020;18(1):256-263. doi:10.1016/j.celrep.2020.02.052

97. Camell CD, Yousefzadeh MJ, Zhu Y, et al. Senolytics reduce coronavirus-related mortality in old mice. Science 2021;373(6552):eabe4832. doi:10.1126/science.abe4832

98. Sepe S, Rosselli F, Cancila V, et al. DNA damage response at telomeres boosts the transcription of SARS-CoV-2 receptor ACE2 during aging. EMBO Rep 2022;23(2):e53658. doi:10.15252/embr.202153658

99. Hoffmann M, Kleine-Weber H, Schroeder S, et al. SARS-CoV-2 cell entry depends on ACE2 and TMPRSS2 and is blocked by a clinically proven protease inhibitor. Cell 2020;181(2):271-280.e8. doi:10.1016/j.cell.2020.02.052

100. Clausen TM, Sandoval DR, Splid CB, et al. SARS-CoV-2 infection depends on cellular heparan sulfate and ACE2. Cell 2020;183(4):1043-1057.e15. doi:10.1016/j.cell.2020.09.033

101. Puray-Chavez M, LaPak KM, Schrank TP, et al. Systematic analysis of SARS-CoV-2 infection of an ACE2-negative human airway cell. Cell Rep 2021;36(2):109364. doi:10.1016/j.celrep.2021.109364

102. Nguyen L, McCord KA, Bui DT, et al. Sialic acid-containing glycolipids mediate binding and viral entry of SARS-CoV-2. Nat Chem Biol 2022;18(1):81-90. doi:10.1016/j.chembiol.2021.09.002

103. Petijean SJL, Chen W, Koehler M, et al. Multipotent 9-O-Acetylated-sialic acid glycoconjugates as potent inhibitors for SARS-CoV-2 infection. Nat Commun 2022;13:2564. doi:10.1038/s41467-022-30313-8

104. WuDunn D, Spear PG. Initial interaction of herpes simplex virus with cells is binding to heparan sulfate. J Virol 1989;63(1):52-58. doi:10.1128/jvi.63.1.52-58.1989

105. Weis W, Brown JH, Cusack S, Paulson JC, Skehel JJ, Wiley DC. Structure of the influenza virus haemagglutinin complexed with its receptor, sialic acid. Nature. 1988;333(6172):426-431. doi:10.1038/333426a0

106. Hulswit RJG, Lang Y, Bakkers MJG, et al. Human coronavirus OC43 and HKU1 bind to 9-O-acetylated sialic acids via a conserved receptor-binding site in spike protein domain A. Proc Natl Acad Sci. 2019;116(7):2681-2690. doi:10.1073/pnas.1809667116

107. Li W, Hulswit RJG, Widjaja I, et al. Identification of sialic acid-binding function for the Middle East respiratory syndrome coronavirus spike glycoprotein. Proc Natl Acad Sci. 2017;114(40):E8508-E8517. doi:10.1073/pnas.1712592114

108. Matsuyama S, Nao N, Shirato K, et al. Enhanced isolation of SARS-CoV-2 by TMPRSS2-expressing cells. PNAS. 2020;117(13):7001-7003. doi:10.1073/pnas.2002589117

109. Limburg H, Harbig A, Bestle D, et al. TMPRSS2 is the major activating protease of influenza A virus in primary human airway cells and influenza B virus in human type II pneumocytes. J Virol. 2019;93(21):e00649-19. doi:10.1128/JVI.00649-19

110. Ziegler CGK, Allon SJ, Nyquist SK, et al. SARS-CoV-2 receptor ACE2 is an interferon-stimulated gene in human airway epithelial cells and is detected in specific cell subsets across tissues. Cell 2020;181(5):1016-1035.e19. doi:10.1016/j.cell.2020.04.035

111. Fodoulian L, Tuberosa J, Rossier D, et al. SARS-CoV-2 receptors and entry genes are expressed in the human olfactory neuroepithelium and brain. iScience. 2020;23(12):101839. doi:10.1016/j.isci.2020.101839

112. Ahn JH, Kim J, Hong SP, et al. Nasal ciliated cells are primary targets for SARS-CoV-2 replication in the early stage of COVID-19. J Clin Invest. 2021;131(13):e148517. doi:10.1172/jci148517

113. Nie J, Li Q, Zhang L, et al. Functional comparison of SARS-CoV-2 with closely related pangolin and bat coronaviruses. Cell Discov. 2021;7(1):1-12. doi:10.1038/s41421-021-00256-3

114. Hui KPY, Ho JCW, Cheung M chun, et al. SARS-CoV-2 Omicron variant replication in human bronchus and lung ex vivo. Nature 2022;603(7902):715-720. doi:10.1038/s41586-022-04479-6

115. Bollavaram K, Leeman TH, Lee MW, et al. Multiple sites on SARS-CoV-2 spike protein are susceptible to proteolysis by cathepsins B, K, L, S, and V. Protein Sci. 2021;30(6):1131-1143. doi:10.1002/pro.4073

116. Zhao MM, Yang WL, Yang FY, et al. Cathepsin L plays a key role in SARS-CoV-2 infection in humans and humanized mice and is a promising target for new drug development. Sig Transduct Target Ther. 2021;6(1):1-12. doi:10.1038/s41392-021-00558-8

117. Lan J, Ge J, Yu J, et al. Structure of the SARS-CoV-2 spike receptor-binding domain bound to the ACE2 receptor. Nature. 2020;581(7807):215-220. doi:10.1038/s41586-020-2180-5

118. Hoffmann M, Pöhlmann S. How SARS-CoV-2 makes the cut. Nat Microbiol. 2021;6(7):828-829. doi:10.1038/s41564-021-00931-x

119. Kliche J, Kuss H, Ali M, Ivashon Y. Cytoplasmic short linear motifs in ACE2 and integrin β3 link SARS-CoV-2 host cell receptors to mediators of endocytosis and autophagy. Sci Signal. 2021;14(665):eabf1117. doi:10.1126/scisignal.abf1117

120. Cantuti-Castelvetri L, Ojha R, Pedro LD, et al. Neuropilin-1 facilitates SARS-CoV-2 cell entry and infectivity. Science. 2020;370(6518):856-860. doi:10.1126/science.abcd2985

121. Daly JL, Simonetti B, Klein K, et al. Neuropilin-1 is a host factor for SARS-CoV-2 infection. Science. 2020;370(6518):861-865. doi:10.1126/science.aba3072

122. Wei C, Wan L, Yan Q, et al. HDL-scavenger receptor B type 1 facilitates SARS-CoV-2 entry. Nat Metab. 2020;2(12):1391-1400. doi:10.1038/s42255-020-00324-0

123. Yeung ML, Teng JLL, Jia L, et al. Soluble ACE2-mediated cell entry of SARS-CoV-2 by interaction with proteins related to the renin-angiotensin system. Cell. 2021;184(8):2212-2228.e12. doi:10.1016/j.cell.2021.02.053

124. Ye G, Liu B, Li F. Cryo-EM structure of a SARS-CoV-2 omicron spike glycoprotein. Nat Commun. 2022;13(1):1214. doi:10.1038/s41467-022-28882-9

125. Meng B, Abdullah A, Ferreira IAM, et al. Altered TMPRSS2 usage by SARS-CoV-2 Omicron impacts infectivity and fusogenicity. Nature. 2022;603(7902):706-714. doi:10.1038/s41586-022-04474-x
116. Malone B, Urakova N, Snijder EJ, Campbell EA. Structures and functions of coronavirus replication–transcription complexes and their relevance for SARS-CoV-2 drug design. Nat Rev Mol Cell Biol. 2022;23(1):21-39. doi:10.1038/s41580-021-00432-z

117. Banerjee AK, Blanco MR, Bruce EA, et al. SARS-CoV-2 disrupts splicing, translation, and protein trafficking to suppress host defenses. Cell. 2020;183(5):1325-1339.e21. doi:10.1016/j.cell.2020.10.004

118. Schubert K, Karousis ED, Joma A, et al. SARS-CoV-2 Nsp1 induces the ribosomal mRNA channel to inhibit translation. Nat Struct Mol Biol. 2020;27(10):959-966. doi:10.1038/s41594-020-0511-8

119. Yuan S, Peng L, Park JJ, et al. Nonstructural protein 1 of SARS-CoV-2 promotes NLRP3 inflammasome activation by RIG-I and MDA5 links epithelial infection to macrophage interferon signaling. iScience. 2021;34(13):108916. doi:10.1038/s41392-020-00438-7

120. Papa G, Mallery DL, Albecka A, et al. Furin cleavage of SARS-CoV-2 ORF3a promotes acute lung injury and mortality in standard laboratory mice. Cell. 2020;183(4):1070-1085.e13. doi:10.1016/j.cell.2020.09.050

121. Chen D, Zheng Q, Sun L, et al. ORF3a of SARS-CoV-2 promotes lysosomal exocytosis-mediated viral egress. Dev Cell. 2021;56(23):3250-3263.e5. doi:10.1016/j.devcel.2021.10.006

122. Thorne LG, Reuschl AK, Zuliani-Alvarez L, et al. SARS-CoV-2 sensing of cGAS-STING and RNA sensing–triggered innate immune responses by SARS-CoV-2 proteins. Sig Transduct Target Ther. 2020;5(1):1-13. doi:10.1038/s41392-020-00515-5

123. Blanco-Melo D, Nilsson-Payant BE, Liu WC, et al. Imbalanced host response to SARS-CoV-2 drives development of COVID-19. Cell. 2020;181(5):1036-1045.e9. doi:10.1016/j.cell.2020.04.026

124. D’Agostillo F, Walters KA, Xiao Y, et al. Lung endothelial and epithelial damage, loss of tissue repair, inhibition of fibrinolysis, and cellular senescence in fatal COVID-19. Sci Transl Med. 2021;13(620):eabj7790. doi:10.1126/scitranslmed.abj7790

125. Thorne LG, Bouhaddou M, Reuschl AK, et al. Evolution of enhanced innate immune evasion by SARS-CoV-2. Nature. 2022;602(7897):487-495. doi:10.1038/s41586-021-04352-y
164. Leppkes M, Bastard P, Human Genetic Effort COVID, et al. Human genetic and immunological determinants of critical COVID-19 pneumonia. Nature. 2022;603(792):587-598. doi:10.1038/s41586-022-04447-0

158. Channappanavar R, Fehr AR, Vijay R, et al. Dysregulated Type I interferon and inflammatory monocyte-macrophage responses cause lethal pneumonia in SARS-CoV-infected mice. Cell Host Microbe. 2016;19(2):181-193. doi:10.1016/j.chom.2016.01.007

167. Junqueira C, Crespo Â, Ranjbar S, et al. Temporal and spatial heterogeneity of host response to SARS-CoV-2 pulmonary infection. Nat Commun. 2020;11(1):6319. doi:10.1038/s41467-020-13017-9

169. Zhang F, Mears JR, Shakib L, et al. IFN-γ and TNF-α drive a CXCL10+ CCL2+ macrophage phenotype expanded in severe COVID-19 lungs and inflammatory diseases with tissue inflammation. Genome Med. 2021;13(1):64. doi:10.1186/s13073-021-00881-3

170. Thakur KT, Miller EH, Glendinning MD, et al. COVID-19 neuropathology at Columbia University Irving Medical Center/New York Presbyterian Hospital. Brain. 2021;144(9):2696-2708. doi:10.1098/rb3.2021.03.007

151. Zhang Q, Bastard P, Human Genetic Effort COVID, et al. Inborn errors of type I IFNs in patients with life-threatening COVID-19. Science. 2020;370(6515):eabc4570. doi:10.1126/science.abd4570

160. Fu J, Kong J, Wang W, et al. The clinical implication of dynamic immunotypes in COVID-19 patients reveals distinct immunotypes with therapeutic implications. Cell Host Microbe. 2021;28(2):222-235.e4. doi:10.1016/j.chom.2021.12.017

159. Mathew D, Giles JR, Baxter AE, et al. Deep immune profiling of SARS-CoV-2 infected patients how SARS-CoV-2 attacks the respiratory and olfactory mucosae but spares the olfactory bulb. Cell. 2021;184(24):5932-5949.e15. doi:10.1016/j.cell.2021.10.027

152. Zhang Q, Bastard P, Liu Z, et al. Inborn errors of IFN-γ immunity in patients with COVID-19. Cell Host Microbe. 2020;28(5):903-912.e2. doi:10.1016/j.chom.2020.09.007

166. Desai N, Neyaz A, Szabolcs A, et al. Temporal and spatial heterogeneity in patients with COVID-19. J Exp Med. 2020;218(10):e20211211. doi:10.1084/jem.20211211

155. Karki R, Lee S, Mall R, et al. ZBP1-dependent inflammatory cell death, PANoptosis, and cytokine storm disrupt IFN therapeutic efficacy during coronavirus infection. Sci Immunol. 2022:eab6294. doi:10.1126/sciimmunol.ab6294

161. Kuri-Cervantes L, Pampena MB, Meng W, et al. Comprehensive characterization of SARS-CoV-2 infection and cytokine shock syndromes. Cell. 2021;184(1):149-168.e17. doi:10.1016/j.cell.2020.11.025

162. Guo Q, Zhao Y, Li J, et al. Induction of alarmin S100A8/A9 mediates activation of aberrant neutrophils in the pathogenesis of COVID-19. J Exp Med. 2021;218(10):e20211211. doi:10.1084/jem.20211211

163. Junqueira C, Crespo Â, Ranjbar S, et al. FcγR-mediated SARS-CoV-2 infection of monocyes activates inflammation. Nature. 2022;606:576-584. doi:10.1038/s41586-022-04702-4

165. Karki R, Sharma BR, Tuladhar S, et al. Synergism of TNF-α and IFN-γ Triggers Inflammatory Cell Death, Tissue Damage, and Mortality in SARS-CoV-2 Infection and Cytokine Shock Syndromes. Cell. 2021;184(1):149-168.e17. doi:10.1016/j.cell.2020.11.025

168. Jiao L, Yang Y, Yu W, et al. The olfactory route is a potential way to evade the central nervous system of rhesus macaques. Cell. 2021;184(24):5932-5949.e15. doi:10.1016/j.cell.2021.10.027

169. Zhang F, Mears JR, Shakib L, et al. IFN-γ and TNF-α drive a CXCL10+ CCL2+ macrophage phenotype expanded in severe COVID-19 lungs and inflammatory diseases with tissue inflammation. Genome Med. 2021;13(1):64. doi:10.1186/s13073-021-00881-3

170. Thakur KT, Miller EH, Glendinning MD, et al. COVID-19 neuropathology at Columbia University Irving Medical Center/New York Presbyterian Hospital. Brain. 2021;144(9):2696-2708. doi:10.1098/rb3.2021.03.007

151. Zhang Q, Bastard P, Human Genetic Effort COVID, et al. Inborn errors of type I IFNs in patients with life-threatening COVID-19. Science. 2020;370(6515):eabc4570. doi:10.1126/science.abd4570

163. Junqueira C, Crespo Â, Ranjbar S, et al. FcγR-mediated SARS-CoV-2 infection of monocyes activates inflammation. Nature. 2022;606:576-584. doi:10.1038/s41586-022-04702-4

165. Karki R, Sharma BR, Tuladhar S, et al. Synergism of TNF-α and IFN-γ Triggers Inflammatory Cell Death, Tissue Damage, and Mortality in SARS-CoV-2 Infection and Cytokine Shock Syndromes. Cell. 2021;184(1):149-168.e17. doi:10.1016/j.cell.2020.11.025

169. Zhang F, Mears JR, Shakib L, et al. IFN-γ and TNF-α drive a CXCL10+ CCL2+ macrophage phenotype expanded in severe COVID-19 lungs and inflammatory diseases with tissue inflammation. Genome Med. 2021;13(1):64. doi:10.1186/s13073-021-00881-3

170. Thakur KT, Miller EH, Glendinning MD, et al. COVID-19 neuropathology at Columbia University Irving Medical Center/New York Presbyterian Hospital. Brain. 2021;144(9):2696-2708. doi:10.1098/rb3.2021.03.007
monkeys. Sig Transduct Target Ther. 2021;6(1):1-11. doi:10.1038/s41392-021-00591-7
187. Matschke J, Lütgethiem M, Hagel C, et al. Neuropathology of patients with COVID-19 in Germany: a post-mortem case series. Lancet Neurol. 2020;19(11):919-929. doi:10.1016/S1474-4422(20)30308-2
188. Muruato A, Vu MN, Johnson BA, et al. Mouse-adapted SARS-CoV-2 protects animals from lethal SARS-CoV challenge. PLoS Biol. 2021;19(11):e1001284. doi:10.1371/journal.pbio.3001284
189. Yang AC, Kern F, Losada PM, et al. Dysregulation of brain and choroid plexus cell types in severe COVID-19. Nature. 2021;595(7868):565-571. doi:10.1038/s41586-021-03710-0
190. Schwabenberg M, Salié H, Tanevski J, et al. Deep spatial profiling of human COVID-19 brains reveals neuroinflammation with distinct microanatomical microglia-T-cell interactions. Immunity. 2021;54(7):1549-1610.e11. doi:10.1016/j.immuni.2021.06.002
191. Muhl L, He L, Sun Y, et al. The SARS-CoV-2 receptor ACE2 of a complete autopsy. Lancet Reg Health – Am. 2020;2(6):100046. doi:10.1016/j.lana.2021.100046
192. Ramani A, Müller L, Ostermann PN, et al. SARS-CoV-2 targets neural- perivascular ‘assembloid’ promotes astrocytic development and enables modeling of SARS-CoV-2 neuropathology. Nat Med. 2021;27(9):1600-1606. doi:10.1038/s41591-021-01443-1
193. Chen J, Li Z, Guo J, et al. SARS-CoV-2 nsp5 exhibits stronger catalytic activity and interferon antagonism than its SARS-CoV ortholog. J Virol. 2022;96(8):e00337-22. doi:10.1128/jvi.00337-22
194. Wu J, Shi Y, Pan X, et al. SARS-CoV-2 ORF9b inhibits RIG-I-MAVS antiviral signaling by interrupting K63-linked ubiquitination of NEMO. Cell Rep. 2021;34(7):108761. doi:10.1016/j.celrep.2021.108761
195. McMahon CL, Staples H, Gazi M, Carrion R, Hsieh J. SARS-CoV-2 protects animals from lethal SARS-CoV challenge. Immunity. 2020;19(11):919-929. doi:10.1016/j.immuni.2021.06.002
196. Owen DR, Allerton CMN, Anderson AS, et al. An oral SARS-CoV-2 neutraliser that has therapeutic potential for COVID-19. Cell. 2021;205(1):80-85. doi:10.1016/j.jneuroim.2008.09.010
197. Ruitenberg MJ, Vukovic J, Blomster L, et al. CX3CL1/fractalkine of nasal immune cell populations and development of tissue-resident SARS-CoV-2- specific CD8+ T cell responses following COVID-19. Nat Immunol. 2022;23(1):23-32. doi:10.1038/s41590-021-01095-w
198. Bryche B, St Albin A, Muri S, et al. Massive transient damage of the olfactory epithelium during SARS-CoV-2 infection predominates in choroid plexus epithelium. Cell Stem Cell. 2020;27(6):937-950.e9. doi:10.1016/j.stem.2020.09.016
199. Wang L, Sievert D, Clark AE, et al. A human three-dimensional neural-vascular ‘assembloid’ promotes astrocytic development and enables modeling of SARS-CoV-2 neuropathology. Nat Med. 2021;27(9):1600-1606. doi:10.1038/s41591-021-01443-1
200. Ramani A, Müller L, Ostermann PN, et al. SARS-CoV-2 targets neurons of 3D human brain organoids. Stem Cell Reports. 2021;16(5):1156-1164. doi:10.1016/j.stemcr.2021.01.016
201. Wang L, Sievert D, Clark AE, et al. A human three-dimensional neural-vascular ‘assembloid’ promotes astrocytic development and enables modeling of SARS-CoV-2 neuropathology. Nat Med. 2021;27(9):1600-1606. doi:10.1038/s41591-021-01443-1
202. Jacob F, Pather SR, Huang WK, et al. Human pluripotent stem cell-derived neural cells and brain organoids reveal SARS-CoV-2 neurotropism predominates in choroid plexus epithelium. Cell Stem Cell 2020;27(6):937-950.e9. doi:10.1016/j.stem.2020.09.016
203. Tiwari SK, Wang S, Smith D, Carlin AF, Rana TM. Revealing tissue-specific SARS-CoV-2 infection and host responses using human stem cell-derived lung and cerebral organoids. Stem Cell Reports. 2021;16(3):437-445. doi:10.1016/j.stemcr.2021.02.005
204. Gomes I, Karmirian K, Oliveira JT, et al. SARS-CoV-2 infection of the central nervous system in a 14-month-old child: A case report of a complete autopsy. Lancet Reg Health – Am. 2021;2:100046. doi:10.1016/j.lana.2021.100046
205. Wang C, Zhang M, García G, et al. ApoE-isoform-dependent SARS-CoV-2 neurotropism and cellular response. Cell Stem Cell 2021;28(2):331-342.e5. doi:10.1016/j.stem.2020.12.018
206. Agnello V, Åbel G, Elfahal M, Knight GB, Zhang QX. Hepatitis C virus and other Flaviviridae viruses enter cells via low density lipoprotein receptor. Proc Natl Acad Sci. 1999;96(22):12766-12771. doi:10.1073/pnas.96.22.12766
207. de Freitas GR, Figueiredo MR, Vianna A, et al. Clinical and radiological features of severe acute respiratory syndrome coronavirus 2 and Zika encephalitis. Eur J Neurol. 2021;28(10):3530-3532. doi:10.1111/ene.14687
208. Lyons DB, Allen WE, Goh T, Tsai L, Barnea G, Lomvardas S. An epigenetic trap stabilizes singular olfactory receptor expression. Cell. 2013;154(2):325-336. doi:10.1016/j.cell.2013.06.039
209. Finlay JB, Brann DH, Abi-Hachem R, et al. Persistent post-COVID-19 smell loss is associated with inflammatory infiltration and altered olfactory epithelial gene expression. Published online April 18, 2022;2022:04.17.488474. doi:10.1101/2022.04.17.488474
210. Roukens AHE, Pothast CR, König M, et al. Neutrophils initiate the destruction of the olfactory epithelium during SARS-CoV-2 infection in hamsters. Published online March 15, 2022;2022:03.15.484439. doi:10.1101/2022.03.15.484439
211. Ruitenberg MJ, Vukovic J, Blomster L, et al. CX3CL1/fractalkine regulates branching and migration of monocyte-derived cells in the mouse olfactory epithelium. J Neuroimmunol. 2008;205(1):80-85. doi:10.1016/j.jneuroim.2008.09.010
212. Bryche B, St Albin A, Muri S, et al. Massive transient damage of the olfactory epithelium associated with infection of sustentacular cells by SARS-CoV-2 in golden Syrian hamsters. Brain Behav Immun. 2020;89:579-586. doi:10.1016/j.bbi.2020.06.032
213. Clara B, Audrey SA, Ophélie AG, et al. Neutrophils initiate the destruction of the olfactory epithelium during SARS-CoV-2 infection in hamsters. Published online April 18, 2022;2022:04.17.488474. doi:10.1101/2022.04.17.488474
214. Bryche B, St Albin A, Muri S, et al. Massive transient damage of the olfactory epithelium associated with infection of sustentacular cells by SARS-CoV-2 in golden Syrian hamsters. Brain Behav Immun. 2020;89:579-586. doi:10.1016/j.bbi.2020.06.032
215. Bryche B, St Albin A, Muri S, et al. Massive transient damage of the olfactory epithelium associated with infection of sustentacular cells by SARS-CoV-2 in golden Syrian hamsters. Brain Behav Immun. 2020;89:579-586. doi:10.1016/j.bbi.2020.06.032
257. Sampson BA, Ambrosi C, Charlott A, Reiber K, Veress JF, Armbrustmacher V. The pathology of human West Nile virus infection. *Hum Pathol.* 2000;31(5):527-531. doi:10.1053/hp.2000.8047

258. Singh S, Metz I, Amor S, van der Valk P, Stadelmann C, Brück W. Microglial nodules in early multiple sclerosis white matter are associated with degenerating axons. *Acta Neuropathol.* 2013;125(4):595-608. doi:10.1007/s00401-013-1082-0

259. Burm SM, Peferoen LAN, Zuiderwijk-Sick EA, et al. Expression of IL-1β in rhesus EAE and MS lesions is mainly induced in the CNS itself. *J Neuroinflamm.* 2016;13(1):138. doi:10.1186/s12974-016-0605-8

260. van Horssen J, Singh S, van der Pol S, et al. Clusters of activated microglia in normal-appearing white matter show signs of innate immune activation. *J Neuroinflamm.* 2012;9(1):156. doi:10.1186/1742-2049-9-156

261. Tröscher AR, Wimmer I, Quemada-Garrido L, et al. Microglial nodules provide the environment for pathogenic T cells in human encephalitis. *Acta Neuropathol.* 2019;137(4):619-635. doi:10.1007/s00401-019-01958-5

262. Ramaswamy V, Walsh JG, Sinclair DB, et al. Inflammasome induction in Rasmussen's encephalitis: cortical and associated white matter pathogenesis. *J Neuroinflammation.* 2013;10(1):918. doi:10.1186/1742-2049-10-152

263. Saifaiyan S, Besson-Girard S, Kaya T, et al. White matter aging drives microglial diversity. *Neuron* 2021;109(7):1100-1117.e10. doi:10.1016/j.neuron.2021.01.027

264. Davalos D, Kyu Ryu J, Merlino M, et al. Fibrinogen-induced perivascular microglial clustering is required for the development of axonal damage in nervous inflammation. *Nat Commun.* 2012;3(1):1227. doi:10.1038/ncomms2230

265. Petersen MA, Ryu JK, Chang KJ, et al. Fibrinogen activates BMP signaling in oligodendrocyte progenitor cells and inhibits remyelination after vascular damage. *Neuron* 2017;96(5):1003-1012.e7. doi:10.1016/j.neuron.2017.10.008

266. Kleinberger G, Brendel M, Mracsko E, et al. The FTD-like syndrome of IL-12 in rhesus EAE and MS lesions is mainly induced in the CNS itself. *J Neuroinflammation.* 2013;10(1):918. doi:10.1186/1742-2049-9-156

267. Zheng M, Gao Y, Wang G, et al. Functional exhaustion of anergic CD8+ T cells in SARS-CoV-2 infection. *Cell Reports Med.* 2020;1(5):100288. doi:10.1016/j.xcrm.2021.100288

268. Espindola OM, Gomes YCP, Brandão CO, et al. Inflammatory cytokine patterns associated with neurological diseases in coronavirus disease 2019. *Ann Neurol.* 2021;89(5):1041-1045. doi:10.1002/ana.26041

269. Heming M, Li X, Räuber S, et al. Neurological manifestations of COVID-19 feature T cell exhaustion and dedifferentiated monocytes. *J Neuroinflammation.* 2021;18(5):533-535. doi:10.1186/s12974-020-01729-2

270. Mahmoudzadeh S, Nozad Charoudeh H, Marques CS, Bhadory S, Ahmadpour E. The role of IL-12 in stimulating NK cells against Toxoplasma gondii infection: a mini-review. *Parasitol Res.* 2021;120(7):2303-2309. doi:10.1007/s00436-021-07204-w

271. Song E, Bartley CM, Chow RD, et al. Divergent and self-reactive immune responses in the CNS of COVID-19 patients with neurological symptoms. *Cell Reports Med.* 2021;2(5):100288. doi:10.1016/j.xcrm.2021.100288

272. Jin WN, Shi K, He W, et al. Neuroblast senescence in the aged brain augments natural killer cell cytotoxicity leading to impaired neurogenesis and cognition. *Nat Neurosci.* 2021;24(1):61-73. doi:10.1038/s41593-020-00745-w

273. Potsoushan C, Darley DR, Wilson DB, et al. Immunological dysfunction persists for 8 months following initial mild-to-moderate SARS-CoV-2 infection. *Ann Immunol.* 2022;23(2):210-216. doi:10.1016/j.ximm.2021.01.0113-x

274. Prudencio M, Erben Y, Marquez CP, et al. Serum neurofilament light protein correlates with unfavorable clinical outcomes in hospitalized patients with COVID-19. *Sci Transl Med.* 2021;13(602):eabi7643. doi:10.1126/scitranslmed.abi7643

275. Kanberg N, Ashton NJ, Andersson LM, et al. Neurochemical evidence of astrocytic and neuronal injury commonly found in COVID-19. *Neurology.* 2020;95(12):e1754-e1759. doi:10.1212/WNL.0000000000010111

276. Virhammar J, Nääs A, Fällmar D, et al. Biomarkers for central nervous system injury in cerebrospinal fluid are elevated in COVID-19 and associated with neurological symptoms and disease severity. *Eur J Neurol.* 2021;28(10):3324-3331. doi:10.1111/ene.14703

277. Al-Aly Z, Xie Y, Rowe B. High-dimensional characterization of post-acute sequelae of COVID-19. *Nature.* 2021;594(7862):259-264. doi:10.1038/s41586-021-03553-9

278. Xie Y, Rowe B, Al-Aly Z. Burdens of post-acute sequelae of COVID-19 by severity of acute infection, demographics and health status. *Nat Commun.* 2021;12(1):6571. doi:10.1038/s41467-021-26513-3

279. Schöll M, Maass A, Mattsson N, et al. Biomarkers for tau pathology. *Mov Cell Neurosci.* 2019;97:18-33. doi:10.1016/j.mcn.2018.12.001

280. Palmqvist S, Schöll M, Strandberg O, et al. Earliest accumulation of β-amyloid occurs within the default-mode network and concurrently affects brain connectivity. *Nat Commun.* 2017;8(1):1214. doi:10.1038/s41467-017-01150-x

281. Braak H, Braak E. Neuropathological staging of Alzheimer-related changes. *Acta Neuropathol.* 1991;82(4):239-259. doi:10.1007/BF00308809

282. Sepulcre J, Schultz AP, Sabuncu M, et al. In Vivo Tau, Amyloid, and Gray Matter Profiles in the Aging Brain. *J Neurosci.* 2016;36(28):7364-7374. doi:10.1523/JNEUROSCI.0639-16.2016

283. Douaud G, Lee S, Alfaro-Almagro F, et al. SARS-CoV-2 is associated with changes in brain structure in UK Biobank. *Nature.* 2022;604:697-707. doi:10.1038/s41586-022-04532-4

284. Altmann T, Tian L, Henderson VW, Greicius MD, Investigators ADNI. Sex modifies the APOE-related risk of developing Alzheimer disease. *Ann Neurol.* 2014;75(4):563-573. doi:10.1002/ana.24135

285. Kuo CL, Pilling LC, Atkins JL, et al. APOE ε4 genotype predicts severe COVID-19 in the UK biobank community cohort. *J Gerontol: Series A.* 2020;75(11):2231-2232. doi:10.1093/gerona/glaa131

286. Graves AB, Bowen JD, Rajaram L, et al. Impaired olfaction as a marker for cognitive decline: Interaction with apolipoprotein E ε4 status. *Neurology.* 1999;53(7):1480. doi:10.1212/WNL.0000000000010111

287. Menachery VD, Graham RL, Baric RS. Jumping species—a mechanism for coronavirus persistence and survival. *Curr Opin Virol.* 2017;23:1-7. doi:10.1016/j.coviro.2017.01.002

288. Temmam S, Vongphayloth K, Baquero E, et al. Bat coronaviruses related to SARS-CoV-2 and infectious for human cells. *Nature.* 2022;16:1-7. doi:10.1038/s41586-022-04532-4

How to cite this article: Natale NR, Lukens JR, Petri WA. The nervous system during COVID-19: Caught in the crossfire. *Immunol Rev.* 2022;311:90-111. doi:10.1111/imr.13114