SARS-CoV-2 infection reaches the human nervous system: How?

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Abstract

Without protective and/or therapeutic agents the SARS-CoV-2 infection known as coronavirus disease 2019 (COVID-19) is quickly spreading worldwide. It has surprising transmissibility potential, since it could infect all ages, gender, and human sectors. It attacks respiratory, gastrointestinal, urinary, hepatic, and endovascular systems and can reach the peripheral nervous system (PNS) and central nervous system (CNS) through known and unknown mechanisms. The reports on the neurological manifestations and complications of the SARS-CoV-2 infection are increasing exponentially. Herein, we enumerate seven candidate routes, which the mature or immature SARS-CoV-2 components could use to reach the CNS and PNS, utilizing the within-body crosstalk between organs. The majority of SARS-CoV-2 infected patients suffer from some neurological manifestations (e.g., confusion, anosmia, and ageusia). It seems that although the mature virus did not reach the CNS or PNS of the majority of patients, its unassembled components and/or the accompanying immune-mediated responses may be responsible for the observed neurological symptoms. The viral particles and/or its components have been specifically documented in endothelial cells of lung, kidney, skin, and CNS. This means that the blood-endothelial-barrier may be considered as the main route for SARS-CoV-2 entry into the nervous system, with the barrier disruption being more logical than barrier permeability, as evidenced by postmortem analyses.

Introduction

A new, highly virulent coronavirus (CoV) capable of infecting humans (HCoV) currently holds much of the world's population hostage. This virus, referred to as SARS-COV-2 and causing the COVID-19 disease in infected subjects, emerged at the end of 2019 in China and the moment is affecting the population in at least 213 countries and territories around the world. This is only the latest in a series of lethal HcoV-caused illnesses, following the emergence of SARS-CoV in 2002 and MERS-CoV in 2012. Within the worldwide storm caused by the COVID-19 outbreak, most patients infected with SARS-CoV-2 and diagnosed with COVID-19 have only mild symptoms or are entirely asymptomatic. Unfortunately, approximately 20% of infected individuals exhibit far more serious symptoms, with 15% being considered "severe" and requiring oxygen, and the remaining 5% being viewed as "critical" and relying on ventilators. Symptoms of these serious cases include signs similar to pneumonia, septic shoock, respiratory failure, and even multi-organ failure. Thus far, an estimated 1-2% of COVID-19 cases have proven to be fatal [1,2], though it must be noted that the majority of fatalities associated with the disease happened in individuals suffering from chronic afflictions, including various cardiovascular diseases, chronic obstructive pulmonary disease (COPD), and other comorbidities [3].

Analyses of deceased SARS-CoV-2 patients have shown that the viral particles reach and are distributed in nervous system tissues (**Table 1**). This discovery begs several important questions: from where did SARS-CoV-2 come to the nervous system and how did it access the brain? What are the brain infection manifestations? Is viral infection persistent in the brain? Are COVID-19 deaths dependent or independent on brain infection? The goal of this work is to get some logical answers to some of these questions.

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To this end, we conducted a comprehensive analysis of existing literature, using the following search strategy and selection criteria. References for this review were collected through searches of PubMed, SCOPUS, and Web of Science for articles published until July 10, 2020. The search terms used were "coronaviruses, SARS-CoV, SARS-CoV-2, 2019-nCov, MERS-CoV, 229E-CoV", and "COVID-19", combined with "nervous system", "neuroinvasion", "neurological manifestation", and "brain." *In vitro* studies on neurotropism potentials of CoV on neural or glial cell cultures were considered. *In vivo* model investigations were included for infection routes (intranasal and intraperitoneal) of neuroinvasion. Post-mortem autopsies and biopsy analyses were considered. Furthermore, clinical findings were searched and included for neurological signs related to CoVs infections.

Although the 2002-2004 outbreak of the severe acute respiratory syndrome (SARS-CoV), as well as the 2012-2020 outbreak of the middle east respiratory syndrome (MERS-CoV) and current COVID-19 are the real newsmakers, it is recognized now that in addition to SARS-CoV, MERS-CoV, and SARS-CoV-2 (all are β-CoVs of the B and C lineage), there are four other coronaviruses (CoVs) capable of infecting humans (HCoVs), which circulate continuously in the human population. These are HCoV-OC43 [4,5] and HCoV-HKU1 [6] (β-CoVs of the A lineage or β1CoVs), and HCoV-229E [7,8] and HCoV-NL63 [9,10] (α-CoVs). Identified in the late 1960s (HCoV-229E and the HCoV-OC43) [11-15] and in 2004-2005 (HCoV-NL63 [16,9,10] and HCoV-HKU1 [6]), these HCoVs are known to be responsible for 3% – 10% cases of the common cold and short-term upper respiratory infections that occur mainly in winter, with a short incubation time [17,18], with about 2% of the human population being healthy carriers of an HCoV [19,20]. Although these HCoV strains can also cause more serious diseases of the lower respiratory tract, such as bronchitis, bronchiolitis, and pneumonia, especially in newborns or infants, elderly people, and immunocompromised patients [19,20], their phenotypes are generally mild, and as a result, these four HCoVs received relatively little attention. Consequently, there is abundant research into CoV that stems all the way back to the 1930s, which has resulted in a considerable knowledge base and various tools for further examining these pathogens in humans.

Data from the *in vitro* experiments on culturing SARS-CoV and SARS-CoV-2 on the cell lines derived from different human and animal organs clearly indicated that there are many similarities as well as differences between these two CoVs. One of the interesting points made in this study was that SARS-CoV-2 (but not SARS-CoV) was able to modestly replicate in the neuronal (U251) cells, which highlighted the potential of this virus to cause neurological manifestations (e.g., confusion, anosmia, and ageusia) in patients with COVID-19 [21]. The same study also showed that the pluripotent stem cell (iPSC)-derived BrainSphere model can be infected with SARS-CoV-2 (SARS-CoV-2/Wuhan-1/2020), which exponentially replicated 10-fold there [22]. The virus particles were found in the neuronal cell body extending into apparent neurite structures. This neural cell model expressed angiotensin-converting enzyme 2 (ACE2, a SARS-CoV-2 receptor on the surface of the host cells interacting with the viral spike (S) protein) but not the transmembrane serine protease-2 (TMPRSS2, the catalytic enzyme responsible for the S protein priming required for the subsequent CoV cell entry), which suggests the presence of alternative proteolytic tools there [22]. This state-of-the-art 3D organotypic cell culture model was already successfully used in the infection studies with the Zika, Dengue, HIV, and John Cunningham (JV) viruses. The most interesting point of these studies was the fact that the functional blood-brain-barrier (BBB) had lost its functionality

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when microglia (which was not derived from the neural precursor cells but from the mesoderm germ layer) invaded the developing brain from the blood, resulting in cytokine release and neuronal damage in models analyzing the infection with HIV and JC viruses [22]. Therefore, one of the reasons why some, but not all, of the patients showed neurological manifestations could be related to the fact that the BBB normally hinders virus entry, but is impaired in some by inflammatory conditions [22].

Are human coronaviruses neurotropic?

Neurotropism of HCoVs represents an interesting problem. Data on the immune-mediated CNS pathology associated with viral infection are traditionally derived from the analysis of mice infected with a member of the *Coronaviridae* family, Murine Hepatitis Virus (MHV) strains, which is a β-coronavirus genetically related to Human CoV-OC4347 [23]. Being a group II coronavirus, MHV represents a natural pathogen of mice that typically infects the liver, gastrointestinal tract, and CNS, and shows various disease manifestations ranging from gastroenteritis to hepatitis and acute and chronic encephalomyelitis [24-28]. It is recognized that there are at least three major mechanisms of the formation of immune-mediated lesions in CNS. They include: a) a systemic inflammatory response syndrome (SIRS) which occurs as a result of an excessive host response to the infection and leads to the dysfunction of various organs, including CNS; b) a direct viral infection of CNS immune cells, such as astrocytes, microglia, and macrophages, leading to the local production of pro-inflammatory cytokines IL-6, TNF-α, IL-1β, and IL12, as well as some toxic agents or subsequent tissue damage via the recruitment and activation of other immune cells and induction of apoptosis [29]; and c) generation of an autoimmune reaction by an adaptive immune response directed against host epitopes or proteins, which are either misrecognized by the pathogen-directed antibodies or expressed by damaged tissues (and previously unrecognized by the adaptive immune system) [30-32]. There is also a possibility for the eventual demyelination caused by immune-mediated events, either through T-cells or by means of other cytokine and chemokine pathways [33].

About 40% (167 out of 417) of COVID-19 patients are known to develop a spectrum of neurological symptoms, such as cerebrovascular diseases, hypoxic/ischemic encephalopathy, impaired consciousness, acute cerebrovascular disease, encephalopathy, acute hemorrhagic necrotizing, corticospinal tract signs, and prominent agitation and confusion(reviewed in [34]). These manifestations prove the presence of a link between the SARS-CoV-2 infection and CNS pathologies and support neurotropism of this virus.

Although the CoVs are not primarily neurotropic viruses and the most published reports defined the respiratory epithelium as their primary target, there is increasing evidence that neurotropism is indeed a common feature of the viruses [35-39]. In addition to the aforementioned MHV, many other members of the β-CoV family have been documented to show neurotropism. This necessitates gaining a clear understanding of whether SARS-CoV-2 can enter the CNS and cause neuronal injury that may result in acute respiratory distress [40] and potentially some other neurological manifestations.

SARS-CoV

Human tissue studies displayed an abundance of ACE2 receptors in the epithelia of the small

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intestine and lung. These receptors were also identified in vesicular systems, such as venous and arterial endothelial cells and arterial smooth muscle cells in all organs studied, including the brain [41]. In agreement with these observations, ACE2 immunostaining was widely distributed throughout the brain in the transgenic mouse (K18-hACE2 model) expressing human ACE2 [42]. A close look at the antigen and viral kinetics of the SARS-CoV virus in transgenic mice revealed that the infection began in the respiratory epithelium, spread rapidly to the alveoli, entered the brain via the olfactory nerve, and progressively invaded cortical and subcortical regions [43,44]. It was also shown that, eventually, the infection extended to several vital brainstem nuclei, such as the nucleus *tractus solitarii*, dorsal motor nucleus of the vagus, and area postrema.. Since the dorsal vagal complex (DVC) is located in the medulla oblongata, the lowest region of the brainstem that controls several autonomic activities, including orchestration of the cardiorespiratory function (heart and breathing) and food intake, injuries of this specific region of the brainstem could be detrimental to the maintenance of homeostasis and explain the cardiorespiratory disorder. Although the animals intracranially inoculated with low-dose virus were characterized by a limited viral spreading, they rapidly succumbed to infection [44]. In animal models, CoV infection was accompanied by a considerable infiltration of lymphocytes and macrophages in the lungs, resulting in a release of proinflammatory cytokines. This occurred in the brain as well as at the pulmonary level, and within 5 days the subject mice entered a lethargic-like state, which would suggest the involvement of the CNS [43,32]. These neuroanatomic seem to point to the idea that the infected organisms die as a result of dysfunction of the cardiorespiratory center in the brainstem [40]. In the past, autopsy results of humans with SARS-CoV infections showed strong evidence of the presence of SARS-CoV by immunohistochemistry, real-time reverse transcription PCR, and electron microscopy [32]. Furthermore, individuals with acute SARS-CoV also exhibited the presence of the virus in cerebrospinal fluid [44].

MERS-CoV

Very limited data are currently available on the neurological disorders and pathology in humans with the MERS-CoV infection. Although the majority of patients infected with MERS-CoV exhibit predominant pulmonary clinical involvement, there are some patients that exhibit neurologic manifestations, such as ataxia, coma, peripheral nerve symptoms and focal motor deficits [32,45,46]. *Ex vivo* analyses of the MERS-CoV infectivity in various human lung cell lines demonstrated that the virus could infect human neuronal lines [47]. In the hDPP4 transgenic mice model, intranasal MERS-CoV inoculation resulted in infection of both lung and brain by the virus at 3-9-day and 7-9-day post inoculation, respectively, indicating different viral infection kinetics [48]. This may indicate a hematogenous infection route. The brain is (possibly) infected via the olfactory nerves, and thereafter infection is rapidly spread to some specific brain areas, including the thalamus and brainstem [44]. Interestingly, the virus particles were detected only in the brain, but not in the lung, in mice infected with low inoculum doses of MERS-CoV, suggesting that the infection in the CNS played a greater role in the high mortality [49]. Similar to SARS-CoV, MERS-CoV is also known to replicate in human macrophages and dendritic cells, lending additional support to the hematogenous hypothesis [50]. The infected model brain consequences included a mild perivascular cuffing [51], and congestion and dilatation of the cerebral vessels and areas of cellular necrosis in the thalamus, hippocampus, and cerebral cortex [32,48].

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SARS-CoV-2 (COVID-19)

Although the SARS-CoV-2 is mainly a respiratory pathogen, it can also manifest neurologically, causing encephalitis and epileptic seizures, which makes CNS involvement likely. The reported neurological sequelae of SARS-CoV-2 further suggest that the neurological impact of the virus needs to be examined. In fact, although the ongoing COVID-19 pandemic is still relatively young, it has already given rise to many neurological and neuroradiological phenotypes, including ageusia, anosmia, Guillain-Barré syndrome, and even acute necrotising haemorrhagic encephalopathy [52-54].

Mao *et al*. recently reported that among the patients with a severe form of COVID-19, more than 88% (78/88) displayed some form of neurologic dysfunction, such as acute cerebrovascular diseases and impaired consciousness [54]. Also, during the current SARS-CoV-2 outbreak, a COVID-19 patient was reported to have lost control over breathing [40]. Since many COVID-19 patients suffer acute respiratory failure, clinicians and healthcare professionals must separate them into cases that are either neurologically affected, or do not display any neurological deficits [55,40]. It would therefore be beneficial to have have a greater understanding of the possible neuroinvasion of the disease, as it can help in the treatment and prevention of respiratory failure related to SARS-CoV-2 [40].

In a recent report it was demonstrated that both human and mouse olfactory sensory neurons do not express the two key genes involved in SARS-CoV-2 entry, namely *TMPRSS243* and *ACE2* [56]. However, the olfactory epithelial support cells and stem cells express both of these genes, similar to nasal respiratory epithelium cells [56]. This suggests that the SARS-CoV-2 infection may possess mechanisms that lead to olfactory dysfunction, and also brings into question whether olfactory bulb can serve as an entry point for CoVs. [56,32]. Since the anosmia symptoms appeared in many SARS-CoV-2 infections, these important questions were raised [34]: Does anosmia represent an indication of a SARS-CoV-2 infection in the CNS, or it is a reflection of an impact on the peripheral nervous system? Furthermore, can the olfactory or optic nerves act as conduits for SARS-CoV-2's entry into the CNS? These questions are also in line with the current lack of published data on human neuropathological manifestations of the SARS-CoV-2 infection.

Neurological manifestations in COVID-19 patients

It is accepted now that the SARS-CoV-2 can reach and be manifested in most human organs and tissues (**Figure 1**). Mao *et al*. (2020) investigated the penetration potential of the virus into the central nervous system in 214 patients [54]. Their results show that 36.4% of the patients had some neurologic abnormalities ranging from some non-specific manifestations, such as headache, seizure, and dizziness to specific manifestations, such as stroke and loss of sense of taste (ageusia) and smell (anosmia) [54]. In fact, gustatory and olfactory dysfunctions are both prevalent in patients with mild-to-moderate COVID-19, despite not having nasal symptoms, whereas some other neurological symptoms and manifestations can be seen in the more severe COVID-19 cases [57-61]. It must be noted here that the more severe neurologic symptoms, such as decreased levels of consciousness, or development of seizures and stroke, were more common in patients in the late stages of the infection, and these symptoms were responsible for the heightened mortality rate in severely affected patients [54].

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It was also hypothesized that CNS infection with involvement and dysfunction of the cardiorespiratory brainstem centers may contribute to the death of infected animals or patients [62,40]. hACE2 transgenic mice that inoculated intracranially or intranasally with virus particles commonly exhibited a disseminated infection of the dorsal vagal complex (area postrema, nucleus tractus solitarius, and dorsal motor nucleus of the vagus) [44]. This complex contains efferent and afferent projections of the vagus nerve to the lungs and respiratory tracts, suggesting that the vagus nerve may also serve as a neuronal route for viral entry into the brain. This leads to the hypothesis that the dysfunction of the cardiorespiratory brainstem centre may be at least partially responsible for the death of CoV infected animals or patients [62,40]. A cytokine storm with excessive levels of proinflammatory cytokines (IL-6, GM-CSF, IL-2, interferon-γ, IL-7, inducible protein 10, TNF-α, macrophage inflammatory protein 1-α, monocyte chemoattractant protein 1, and monocyte chemoattractant protein 1) may also contribute to the lethality of the COVID-19 infection [63,64]. This is illustrated by recent reports of a COVID-19 patient with an acute necrotizing encephalopathy, a rare complication observed in infections with viruses such as influenza, and related to a cytokine storm in the brain without direct viral invasion [52,61].

A post-mortem histological analysis of the brain of a 71-year-old man who died from complications of COVID-19 revealed the presence of several types of pathological lesions, such as a widespread hemorrhagic and lesion of white matter with a clusters of macrophages, necrotic blood vessels and perivascular inflammation, acute axonal injury, demyelination, a marked lesions of central axonal injury, associated extravasated blood, and surrounding myelin loss [65]. Despite all of these dramatic neurological manifestation in this patient, a routine histological examination of the olfactory bulb/nerve revealed only aging-related corpora amylacea [65]. More globally, the presence of brain tissue edema and partial neuronal degeneration were reported in autopsy reports of deceased COVID-19 patients [66].

SARS-CoV-2 cellular entry receptors

It appears that all the major requirements for efficient hijacking of the nervous cells/tissues by the SARS-CoV-2, which caused the COVID-19 outbreak, are in the place. This includes utilization of the ACE2 (which is present on the surfaces of the cells in a wide variety of human tissues, including the brain) as a cellular entry receptor and the presence of the spike glycoprotein possessing affinity for ACE2, which is ~10-20 fold higher than that of the SARS-CoV spike protein [67,68]. All this indicates that SARS-CoV2 may have higher neuroinvasive potential compared to previous HCoVs. It was also shown that the SARS-CoV-2 receptor ACE2 is expressed in endothelial cells of cerebral capillaries, and within the brain parenchyma in both neurons and microglia [69]. However, there is no complete expression profile of the catalytic enzymes that are required for CoV entry, such as transmembrane serine protease 2 (TMPRSS2) and Furin, on the surface of the nervous tissue cells, from where the COVID-19 can inter to the human nervous system.

Recent studies showed that a subset of COVID-19 patients exhibit altered olfactory function [70-72]. Single-cell and bulk RNA-Seq datasets from human nasal biopsy [73] were analyzed to identify the cell types in the human olfactory neuroepithelium (which is an extracranial site supplying input to the olfactory bulbs of the brain) and in the olfactory bulb that express cell entry molecules (ACE2 and TMPRSS2, as well as Furin) that mediate infection by SARS-CoV-2 [56]. This was further complemented by the analysis of the single-cell RNAseq data

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from whole mouse olfactory bulb from juvenile mice (age postnatal day 26-29) [74] as well as single-cell RNAseq data from the olfactory bulb from the adult male mice (8–12 weeks-old) [56]. These analyses revealed that two key genes involved in SARS-CoV-2 entry, namely ACE2 and TMPRSS2, were expressed in the samples from the whole olfactory mucosa in mouse and human in addition to the thiol proteases cathepsins Ctsb and Ctsl [56]. However, neither olfactory sensory neurons nor olfactory bulb neurons expressed these genes (with the exception of cathepsins Ctsl), which instead were expressed in several stem, perivascular, and support cells [56]. Such results suggest that anosmia and related disturbances in odor perception in COVID-19 patients could be associated with the SARS-CoV-2 infection of non-neuronal cell types [56].

Vavougios proposed that the furin-like cleavage site of the CoV spike protein could be an important determinant for the neurotropism of this virus (i.e., its ability to infect nerve tissue) [75,76]. In fact, it was found that cleavage of the S-protein by furin or furin-like proteases is important for the invasion and virulence of SARS-CoV and MERS-CoV [77]. Furthermore, the proteases determine the host tissue tropism and specificity of these CoVs [77], letting them infect the nervous system via membrane fusion. However, additional studies are necessary to determine if the furin-like cleavage site on the spike protein of SARS-CoV-2 plays a certain role in its invasion of the nervous system. Another important issue that also requires careful future analysis is the presence and sustainability of the nervous system damage after the cure of the COVID-19 infection. This became especially troublesome in light of the fact that the anosmia and ageusia, which are frequently observed among COVID-19 patients, also serve as characteristic and prodromal non-motor manifestations of Parkinson's disease [78,79].

It is also possible that other SARS-CoV-2 receptors may exist, or another cellular entry mode is utilized by SARS-CoV-2 for hijacking the nervous cells/tissues. These possibilities were supported by [80], whose analysis suggested the presence of a different receptor repertoire potentially involved in the SARS-CoV2 infection at the epithelial barriers and in the immune cells, such as the co-expression of ACE2, CD147 (BSG), and CD26 (DPP4). Changes in the expression of these receptors related to gender, age, smoking, and obesity, as well as to the status of the disease, may further contribute to COVID-19 severity and morbidity patterns [80]. Using a combination of structural and molecular modelling approaches, Fantini *et al*. (2020) revealed that the sialic acids linked to host cell surface glycoproteins and ganglioside can also serve as an additional cellular entry route for SRSR-CoV-2 [81], similar to influenza virus, SARS-CoV, and HCoV OC43 [82,83]. A new type of ganglioside-binding domain (111–158) at the tip of the N-terminal domain of the SARS-CoV-2 S protein was identified, which is fully conserved among clinical isolates worldwide, and sialic acid and ganglioside bind chloroquine with high affinity [81].

Although there is a 77% sequence identity between SARS-CoV and SARS-CoV-2, Hassanzadeh *et al*. (2020) discovered that the SARS-CoV-2 S protein has a slightly higher positive charge than SARS-CoV. This is because it has five less negatively charged residues andfour more positively charged residues, which may be why the protein has a higher affinity for negatively charged regions of other molecules in both specific and non-specific interactions [84]. Analysis of the peculiarities of the S protein binding to the host ACE2 receptor showed a 30% higher binding energy for SARS-CoV-2 than the SARS-CoV S protein [84]. Therefore, SARS-CoV-2 is expected to have higher efficiency than SARS-CoV in reaching the brain after entering through the cells [84].

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Cigarette smoking, COVID-19 infectivity and neurotropism

Cigarette smoke has been shown to increase patient susceptibility to COVID-19, with smokers suffering from the disease being far more likely to develop critical illnesses [85-87]. One study of 1,099 COVID-19 patients revealed that only 4.7% of non-smokers required mechanical ventilation, were admitted to an intensive care unit (ICU), or died, compared to 12.3% of smokers [1]. Although the exact mechanism for such an association is uncertain, one of the potential explanations can be found in the fact that cigarette smoke can increase the levels of ACE2 expression in the lungs of mammals [88]. In the case of SARS-CoV infection, ACE2 levels may influence the progression of the disease: within a group of mice engineered to express human ACE2, mice with the highest levels of ACE2 mRNA displaying the shortest survival time after being exposed to SARS-CoV [43].

In agreement with this model, analysis of the datasets of large, small, and bronchial airway epithelium of current and former smokers revealed a noticeable upregulation of pulmonary ACE2 gene expression in all datasets of smokers compared to non-smokers, irrespective of tissue subset [89]. Another study also showed that ACE2 expression in the lower airways is upregulated by active cigarette smoking and COPD, which might help explain the higher risk of serious COVID-19 in patients that smoke [90]. Interestingly, in this study, smoking status was significantly related to the levels of the ACE2 gene expression in the airways of these participants, where current smokers showed a significantly higher gene expression than never-smokers, whereas former smokers' levels were between current and never-smokers [90]. This ACE2 overexpression in human bronchial epithelial cells is mediated by nicotine exposure specifically through the α7 subtype of nicotine acetylcholine receptors (α7-nAChR) [91], which was significantly correlated with expression of *CHRNA7* gene encoding the α7-nAChR. The levels of the *CHRNA7* expression were also correlated with the body mass index, raising an intriguing scenario, where the nicotine receptor mediation of ACE2 may also be related to the high proportion of obese individuals among the COVID-19 cases [92]. Further support is given by the fact that cigarette smoke might cause a dose-dependent upregulation of ACE2, in both rodent and human lungs [93].

There are also reports indicating that smoking may result in higher levels of androgren hormones like testosterone. The androgen receptor has been shown to increase the expression of TMPRSS2 [94], and sex steroid modulation of TMPRSS2 serves as a possible mechanism that may explain the differences in SARS-CoV-2 infection rates between females and males [95]. In line with these observations, RNA-sequencing data analysis for lung and oral epithelial tissues of human COVID-19 patients clearly demonstrated that both TMPRSS2 and ACE2 were significantly upregulated among smokers versus non-smokers [96]. It was also found that there was a correlation between the smoking-mediated upregulation of the androgen pathway and the upregulation of ACE2/TMPRSS2 expression, and that the androgen receptor gene and ADAM17 (a key mediator of ACE2 activity) were upregulated in smokers [96]. Furthermore, smoking was shown to induce furin upregulation, although to a lesser degree than ACE2 [89].

Taken together, these observations support the idea that epithelial cells may be more susceptible to the COVID-19 virus as a result of smoking [96]. But what one can say about smoking and neurotropism of CoVs? ACE2 expression in human brain vessels was significantly elevated by cigarette smoke extract (CSE) treatment. Furthermore, it was found that ACE2 expression is increased in vessels exposed to diabetes or smoking and in ischemic brains , which leads to them being more susceptible to infection. Also, ACE2 expression was upregulated in primary cultured human blood vessels with diabetes when compared to healthy

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vessels [97]. Therefore, the regulation of ACE2 expression by cigarette smoke in the brain likely has a significant effect on SARS-CoV-2 susceptibility, and might facilitate viral dissemination [93]. The harmful effects of CSE on the BBB can upregulate several genes related to inflammation, such as *VCAM1* and *ICAM1*, which also have destructive effects on the BBB [97].

Nervous system access routes of HCoVs and related pathophysiology

Coronaviruses (CoVs) are primarily not neurotropic viruses, and their primary target is the respiratory epithelium. However, although the angiotensin-converting enzyme-2 (ACE2), which serves as a major receptor for Severe Acute Respiratory Syndrome coronavirus (SARS-CoV) and SARS-CoV-2, and which is an enzyme attached to the cell membranes of cells in the arteries, lungs, kidney, heart, and intestines, it can also be found in glial cells in spinal and brain neurons [98]. SARS-CoV and SARS-CoV-2 can therefore use these receptors to enter, attach, multiply, and damage the neuronal tissue. Studies on mice also show that SARS-CoV can enter the brain through the cribriform bone or through a retrograde transfer via the olfactory epithelium, and in seven days can reach the brain. The virus can also enter the brain directly due to a disruption of the BBB during the viremia phase of the disease. The invasion of peripheral nerve terminals by CoV is another postulated mechanism, after which the virus enters the CNS through the synapse connected route. Given that SARS-CoV-2 is very similar to SARS-CoV, it is likely that it can invade the CNS using the same methods as SARS-CoV.

However, though the receptor's expression pattern can determine which cells can be infected, not all cells that express the receptor, or even cells with the highest receptor expression, are necessarily the primary targets of the viral attack. This can be exemplified by the mouse hepatitis virus (MHV) studies, where the MHVR receptor is highly expressed in the liver, but barely so in neurons. On the other hand, the MHV strain JHM.SD, which is highly neurovirulent, is not able to replicate in the liver during viral infection, [99]. Mapping the viral tropism *in vivo* and the virulence factors that contributed to pathogenesis required considerable time and energy. Surprisingly, it was revealed that tissue tropism was not solely impacted by the viral spike protein, but instead by other viral "background genes," such as replicase and nucleocapsid, in addition to different viral accessory genes, in which can also be used for the determination of tropism (reviewed in [100]). Pathogenesis can therefore not be directly inferred from the knowledge of the receptor and spike protein alone. Future studies on SARS-CoV-2 will define tissue tropism and whether it parallels SARS-CoV or not [101].

Let us look more closely on the potential routes for SARS-CoV-2 entry into the CNS (see **Figure 2**). The observation of the presence of the viral-like particles in brain capillary endothelium and their active budding across the endothelial cells strongly suggested that the hematogenous route and the endothelial bed were the most likely pathway to the brain [102]. Expression of the human receptor ACE2, which serves as a receptor and the binding target for the trimeric spike protein of SARS-CoV-2, by the vascular endothelium [41] also supported this interpretation [102]. However, other routes of the SARS-CoV-2 CNS entry, such as retrograde axonal transport from the olfactory bulb, cannot be ruled out. In line with this idea, there is experimental evidence showing the capability of neuroinvasion by HCoV-OC43 and SARS-CoV in mice infected intranasally with these viruses [64]. It was hypothesized that this could happen as a result of a disruption of the nasal epithelium and the resulting neuronal

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dissemination of the virus [64]. This idea would explain the onset of early signs of anosmia as a precursor to other neurological symptoms. Furthermore, based on the previously observed ability of other viruses such as SARS-CoV in the brainstem to induce the dysfunction of the cardiorespiratory center [44], it was hypothesized that the respiratory failure of COVID-19 patients may be governed by the neuroinvasive potential of SARS-CoV-2[40,102]. Therefore, COVID-19 might cause respiratory failure and death not through damage to the lungs, but by affecting the brain.

Baig *et al*. have suggested [55] that the SARS-CoV-2 may access the brain using the same "transcribrial route" defined for other pathogens that target the CNS (such as *Naegleria fowleri* causing meningoencephalitis) [103] and for the delivery of drugs and embryonic stem cells to brain [104]. It is also likely that the SARS-CoV-2 dissemination across the cribriform plate of the ethmoid bone or in the systemic circulation may result in cerebral involvement, similar to what was reported in patients affected by SARS-CoV [44]. As per Baig *et al*., by virtue of its presence in general circulation, SARS-CoV-2 can be passed into cerebral circulation, where it will have a chance to interact with ACE2 expressed in the capillary endothelium, and thereby to infect cells there, causing damage to the endothelial lining and providing viral access to the brain [55]. Importantly, SARS-CoV-2 interaction with the ACE2 receptors expressed in neurons can lead to virus entry and begin a cycle of viral budding and neuronal damage without substantial inflammation, as was reported previously in SARS-CoV cases [44]. It should be noted that well before the anticipated neuronal damage occurs, the endothelial ruptures in cerebral capillaries, together with bleeding within the cerebral tissue, can be fatal in COVID-19 patients [55]. SARS-CoV-2's movement to the brain via the cribriform plate located near the olfactory bulb can act as an additional path that would allow the virus to reach and affect the brain. All this clearly indicates that the observation of hyposmia or an altered sense of smell in an early and uncomplicated stage of a COVID-19 patient should be thoroughly investigated for CNS involvement [55].

1. Olfactory route

There is a growing interest in the study of the olfactory epithelium (OE). It is the most proximal axonal area of the human brain, with neurons that can be regenerated. Many studies show a strong link between olfactory deficiency (for example, loss of smell) and neurodegenerative diseases such as (Alzheimer's and Parkinson's diseases, AD and PD, respectively). The main neurological manifestation of COVID-19 is the loss of taste or smell. Since most instances of smell loss occur without significant rhinorrhea or nasal congestion the virus likely targets the chemical senses in ways that are different from those utilized by other common cold-causing agents or endemic coronaviruses [70]. Therefore, it seems that the olfactory route represents a logical pathway of SARS-CoV-2 entry into the CNS. In fact, analysis of SARS-CoV-2 prevalence in clinical specimens showed that the viral copy number found in nasal swabs is ~200 fold higher than those found in the bronchoalveolar lavage or pharyngeal swabs [105,106]. The utilization of the olfactory route is further supported by the fact that issues with smell have been reported internationally, reporting a prevalence as high as 85% in a large, multicenter European survey [107]. Furthermore, high intensity ACE2 staining was detected in olfactory mucosal biopsies, with a 200-700 fold ACE2 enrichment in the olfactory neuroepithelium (sustentacular cells) relative to the nasal respiratory or tracheal epithelial cells [108]. This cellular tropism of SARS-CoV-2 may underlie its high transmissibility and

association with dysfunction of olfactory neuroepithelium receptors in the nasal and oral mucosa, and also suggests the existence of a viral reservoir that may be a good candidate for intranasal therapy. On the other hand, ACE2 was not found in immature and mature olfactory neurons [108]. Taken together, these observations of the enhanced expression of ACE2 localized to the olfactory neuroepithelium of the human airway suggests that COVID-19 infection and replication may take place in the apical layer of nasal and olfactory mucosa, resulting in olfactory loss and acting as a possible entry point of the virus into the CNS, causing neurological symptoms [54,108]. Furthermore, although the post-mortem examination showed no inflammatory infiltrates or neuronal necroses in the brains of the deceased COVID-19 patients analysed histologically, and although the SARS-CoV-2 RNA copy numbers were predominantly low in the brain, the corresponding values detected in the olfactory bulb were higher than those in the brainstem, supporting the hypothesis of the viral entry into the brain via the lamina cribrosa [109]. In light of the facts that the swabs from olfactory sustentacular cells bear ~200-fold SARS-CoV-2 RNA copy numbers compared to those found in the bronchoalveolar lavage, and that the olfactory sustentacular cells express 200-700-fold more ACE2 relative to the nasal respiratory or tracheal epithelial cells, a fundamental question arose: are there local structures responsible for the COVID-19-associated loss of smell and taste? This seems to be the case, since the microenvironment becomes favourable for the release and/or recruitment of inflammatory leukocytes and cytokines, and subsequently acute reversible or chronic impairment of these chemosensory functions [70,110,111].

2. Blood-nervous system barriers (BNSBs)

Humans have evolved highly sophisticated barrier systems to prevent the entry of potentially harmful substances into the nervous system. The CNS contains four types of such barriers, which are the blood-brain-barrier (BBB), the choroid plexus (CP, which is a vascular tissue found in all cerebral ventricles that produces the cerebrospinal fluid (CSF) of the CNS) blood-cerebrospinal-fluid-barrier (BCSFB), the meningeal-brain-barrier (which consists of the three membranes that envelop the brain and spinal cord, with the meninges in mammals being the dura mater, the arachnoid mater, and the pia mater), and the lymphatic vessel brain barrier. In addition, there is one more barrier of the peripheral nervous system, namely, the blood-nerve barrier. However, some viruses are able to directly manipulate the BBB or BCSFB to enter the CNS, whereas others hijack host immune cells or travel within peripheral nerves. The blood-nervous system barrier (BNSB) may represent the main route of the SARS-CoV-2 for breaching the nervous system (CNS and PNS).

The nervous system has barriers that isolate it from the bloodstream and help it achieve the complex microenvironment control necessary for complex neural signalling. Although all these main physiological nervous barriers differ in location, size, morphology, and function, their main structural units are the epithelial or endothelial cells, which are known to express both ACE2 and TMPRSS2. The vascular endothelial cells constitute the interface between the interstitial fluid of the CNS tissue and the blood. The blood-cerebrospinal fluid barrier (BCSFB) comprises a single layer of endothelial/epithelial cells at the choroid plexus or meninges. It is a fluid–brain barrier consisting of two membranes that separate blood from CSF at the capillary level, and CSF from brain tissue. Epithelial cells separate the plexus or meningeal blood from the CSF. The BCSFB regulates most of the exchange of ions, water, and other substances that can be found between blood and CSF. A few localized brain regions, such as the pineal and the area postrema, are called circumventricular organs (CVOs) and lack the vascular BBB, but rather have a barrier of ependymal cells between the CSF and CVO tissue, and of tanycytes between adjacent brain tissue and the CVO. Therefore, the BCSFB represents

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a regulatory interface comprising a monolayer of cells that separates the blood from the fluids of the CNS [112,113].

As most neurological disorders in COVID-19 patients are demonstrated in somewhat aged patients, the well-documented effects of age on the endothelial/epithelial barriers and specifically of the BNSB should be kept in mind. The exponential decline of integrity/permeability of these barriers is linked to age as reviewed in detail by Delaney and Campell [114]. It is becoming increasingly evident that the pericytes are susceptible to age-dependent deterioration at the BBB. Breakdown of the paracellular pathway, pericyte loss, and transcellular permeability can exacerbate the events linked with age, and can lead to the extravasation of blood-borne material. The susceptibility of endothelium towards many intrinsic destructive agents is known to increase with aging [114]. Again, these facts may provide some explanations of why the SARS-CoV-2 particles were detected in all post-mortem nervous tissue biopsy examined (see **Table 1**).

2.1. The blood-brain barrier (BBB) route

The BBB, located in the brain microvessels, is the largest brain barrier in terms of length, (close to 650 km) and surface (10–20 m^2). It protects the brain from exogenous and circulating threats and maintains brain homeostasis [115]. Despite its size, various neuroinvasive viral pathogens, such as rabies, HIV-1, West Nile, Zika, and influenza are able to breach the BBB [116-125]. These viruses negatively affect the barrier by direct interaction with the endothelial cells, as well as by induction of host immune responses that result in elevated expressions of pro-inflammatory chemokines, cytokines, and cell adhesion molecules that lead to a deterioration of the barrier's functional and structural integrity [126]. A disruption of the BBB can result in the crossing of viral particles and infected immune cells, which can further elevate the levels of inflammatory mediators [126-129]. It is therefore possible that SARS-CoV-2 can use these mechanisms of neuroinvasion, and may also primarily enter the CNS by crossing the BBB. Interactions between SARS-CoV-2 and components of the BBB therefore have the potential to significantly impact neuropathogenesis. Further support to the BBB route hypothesis is given by the facts that BBB is disrupted in hypertension [130] and hypertension is a frequent comorbidity for COVID-19 [131-135].

The BBB is a highly restrictive barrier that protects the CNS from aberrant immune responses and pathogens in the periphery. BBB is formed by the brain endothelial cells lining the cerebral microvasculature with about 50–100 times tighter contacts than that in the peripheral microvessels and astrocytes, which are in direct contact with the endothelial cells [136]. Astrocytes play a central role in maintaining homeostasis within the CNS by regulating the integrity of the BBB, as well as by controlling the uptake of excess neurotransmitters and other extracellular factors that may perturb neurotransmission [136]. It was also pointed out that several biomolecules, such as Endothelin-1 (ET-1), Glutamate, IL-1β, IL-2, IL-6, TNFα, Macrophage inflammatory proteins MIP-2, and nitric oxide might modulate the BBB permeability, with at least some of these biomolecules being released by astrocytic glial cells [136].

Notably, a deregulated immune response serves as an important mediator of COVID-19 mortality [137], as critical illnesses are more likely to develop in patients with heightened levels of inflammatory cytokines [138-143]. These conditions are referred to as ‘‘cytokine storms’’ and result in an increase in vascular permeability, which facilitates immune cell efflux into affected tissues, while also possibly worsening pneumonia [144]. Importantly, most of the proteins that were shown to modulate the BBB permeability [136] are part of the cytokine storm

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in severe COVID-19 cases. One of the outputs of the systemic inflammation is known to cause vascular injury, including breakdown of collagen and permeability of BBB. For example, influenza A virus infection disturbs BBB via the systemic elevation of the levels of the matrix metallopeptidase 9 (MMP-9) [145-147], which is a member of the family of zinc-metalloproteinases involved in the degradation of the extracellular matrix and which breaks collagen present in the basal membrane of every arterial wall, thereby leading to a high collagen turnover in systemic circulation [148] and to the increase in BBB permeability. Such BBB permeability elevation represents a link between MMPs (specifically MMP-9) and CNS disorder [149,150].

Under physiologic conditions, the BBB is relatively impermeable, though recently the list of biomolecules capable of modulating the permeability, integrity, and tightness of the BBB was extended by inclusion of the SARS-CoV-2 spike protein [151]. It was shown here that introduction of the viral spike proteins into the model systems recapitulating the essential features of the BBB resulted in a breach of the barrier. Furthermore, SARS-CoV-2 spike protein was shown to increase the *MMP3*, *CCL5*, *CXCL10*, *ICAM-1*, and *VCAM-1* (which are cell adhesion molecules, CAMs) gene expression levels, alter mRNA levels of interleukins IL-1β and IL-6, and trigger a pro-inflammatory response on brain endothelial cells that may further contribute to an altered state of BBB function [151]. These observations were used to support a hypothesis that SARS-CoV-2 is potentially a neuroinvasive virus since it can turn on the machinery to enable the migration of infected immune cells into the brain parenchyma [151]. In blood vessels, the increase of *VCAM1 and ICAM1* in response to the pro-inflammatory cytokines plays a crucial role in the adhesion of leukocytes, including macrophages and neutrophils, with the end result being disruption of the BBB and inflammation of the brain [97]. Viral gene products can also contribute to the BBB breakdown through up-regulation of many biomarkers [152]. *CXCL8*, *CXCL10*, *CXCL13*, *VCAM-1*, *MMP2*, *MMP14*, and *IL-6* were shown to be over-expressed in COVID-19 patients [153]. All of these molecules were demonstrated to increase the permeability of the endothelial/epithelial cell barriers (especially in nervous system) or decrease its electric resistance and/or cleave the tight junction proteins and promote leukocyte extravasation from the blood (reviewed in detail in [152]).

Therefore, SARS-CoV-2 is able to breach the BBB during the course of ongoing infection. Then, similar to the earlier observations for SARS-CoV [154], interactions of SARS-CoV-2 S protein with ACE2 in multiple brain regions allows the virus to infect the brain. More severe cases of COVID-19 may result in higher probabilities of BBB disruption, which can be associated with strong immunologic responses, such as the cytokine storm pathologies or some co-infection, or other comorbidities.

Therefore, viruses might invade the CNS by entering through the endothelial cells of the BBB and the blood–CSF barrier in the choroid plexus. Studies conducted by Bulfamante *et al*. [155] and Paniz-Mondolfi *et al*. [102] strongly support this hypothesis. The authors captured the viral particles using a cytoplasmic vacuole at the endothelial neural cell interface in a transmission electron microscope, suggesting that SARS-CoV-2 is able to bind to vascular endothelium, penetrate the BBB, and invade nervous tissues through hematogenous pathways.

The presence of SARS-CoV-2 in the brain was demonstrated by transmission electron microscopy analysis of the sections obtained at post-mortem that revealed the presence of 80 to 110 nm viral particles in frontal lobe brain sections [102]. The presence of the virus there was further confirmed by testing the frozen front lobe tissue via running in parallel brain samples in 4 RT-PCR assays that targeted different regions of the viral genome, *ORF1/a* and *E* genes, *N1*, *N2*, *N3*, *N2* and *E* genes, and *ORF1ab* and *S* genes. SARS-CoV-2 was detected in the brain tissue, while the RT-PCR testing did not detect SARS-CoV-2 in a post-mortem

cerebrospinal fluid (CSF) samples [102]. However, other reports detected SARS-CoV-2 in the CSF samples of the COVID-19 patients from three different countries (USA, Brazil, and Japan).

In the first report, PCR detected the SARS-CoV-2 in the CSF of a 40-year-old Los Angeles resident with type 2 diabetes mellitus and obesity, who developed fever and temporary loss of consciousness (syncope) and was admitted for encephalitis [156,157]. In the second report, the SARS-CoV-2 genome was detected and sequenced in a 42 year-old resident of São Paulo with suspected demyelinating disease [158]. In the third report, RT-PCR analysis detected SARS-CoV-2 in the CSF of a Japanese patient with meningitis/encephalitis associated with SARS-CoV-2 [159]. Although SARS-CoV-2 RNA was found in the CSF, no reports have detected and/or demonstrated the presence of the viral particles in the CSF of COVID-19 patients. Therefore, the RT-PCR positivity of the CSF samples for the SARS-CoV-2 RNA does not necessarily imply the presence of the entire infectious viral particles in there, as clearly demonstrated by the inability to detect the full-genome consensus in the CSF samples, where only 1580-nucleotides of two fragments from ORF1a were sequenced [158]. Despite all this, the data collected so far support the BBB breach as an important SARS-CoV-2 entry route. Furthermore, it is possible that the SARS CoV-2 infection could be more persistent in the CNS, which is clearly an immunoprivileged site [158]. Another possibility for the SARS-CoV-2 to cross the BBB and pass into the CNS is via the infection of the blood cells capable of BBB crossing [160].

Observation of the virus-like particles in blood vessel endothelial cells of BBB may point to a hematogenous route of entry of the virus into the nervous system [102]. Neuronal retrograde and hematogenous routes were considered for the entry of neurotropic respiratory viruses into the CNS [64]. In the hematogenous route, viruses gain access to the CNS by using inflammatory cells as “Trojan horses”, or by infecting endothelial cells of the BBB or epithelial cells of the blood-cerebrospinal fluid barrier in the choroid plexus [64]. In the neuronal retrograde route, viruses undergo retrograde axonal transport to reach the neuron cell bodies in the peripheral and or central nervous system [64]. For example, analysis of the MERS-CoV tissue pantropism (i.e., the ability of a virus to indiscriminately affect many kinds of tissues) has shown that MERS-CoV can enter the bloodstream after endothelial infection *in vivo* [161]. This hypothesis is further supported by the presence of SARS-CoV-2 in the CSF fluid of a COVID-19 patient presenting viral encephalitis [162,61].

2.2. The blood-nerve barrier (BNB)

The mammalian blood-nerve barrier (BNB) is the second most restrictive vascular system after the BBB [163]. Peripheral nerves are structurally divided into three compartments: **epineurium**, where fenestrated macrovessels directly derived from the extrinsic peripheral nerve blood supply are located; the inner **perineurium**, which surround the **innermost endoneurium** compartment of the peripheral nerve. The BNB, formed by the tight junction-forming microvessels within peripheral nerve endoneurium, allows for effective axonal signal transduction. The restricted permeability of this barrier protects the endoneurial microenvironment from drastic concentration changes in the vascular and other extracellular spaces. This barrier supplies cover to the nerves everywhere in human body, constitutes the endothelial cells, and is characterized by very compact structure (**Figures 2D1** and **2D2**). The analysis of its transcriptome provided insights into the mechanisms of microbial entry from the bloodstream into peripheral nerves, human BNB response to injury, and response to viral infections.

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Inflammatory and metabolic diseases, as well as traumatic lesions of the nervous system, are accompanied by BNB/BDB (blood dorsal ganglion barrier) opening. Opening of the BNB (or permeable/leaky BNB) can be the first sign preceding neuropathic pain, which synchronises with many agents, such as cytokines, growth factors, and microRNAs [164]. Because endothelial cells forming the BNB are the only cells that come into direct contact with the blood constituents in the PNS, endothelial cells can be easily manipulated via system circulation, or indirectly via pericytic activity, including release of various cytokines and chemokines that influence endothelial function [163]. The BNB endothelial cells could transport the IgG, RNA, chemokine, hormones, and delivery drugs, while the large molecular weight antibody subclasses (sIgM and sIgA) do not undergo human BNB transport under standard physiological condition, which may modulate in pathophysiological conditions. BNB endothelial cells respond to physiological cytokine/chemokine stimulus and normal/pathologic leukocyte trafficking across the BNB [165].

Some neurotropic viruses have been found to be able to hijack the peripheral nerve barrier, such as herpes simplex virus and swine hemagglutinating encephalomyelitis virus (HEV) [37,166,167]. Although there is no data concerning SARS-CoV-2 trafficking across the BNB, some observations concerning the barrier permeability may point to the ability of SARS-CoV-2, or at least its proteins, to target the BNB and to modulate its peripheral nerve immunosurveillance in COVID-19 pathogenesis. In fact, several disorders, such as Guillain–Barré syndrome (GBS), chronic inflammatory demyelinating polyradiculoneuropathy (CIDP), acute inflammatory demyelinating polyradiculoneuropathy (AIDP), and vasculitic neuropathy (VN) are characterized by hematogenous leukocyte infiltration (predominantly monocytes and T-cells) into peripheral nerves via the BNB, with resultant demyelination and axonal degeneration [54,59,60,58,61,138,160]. Therefore, it seems that leukocyte extravasation alone can impact junctional protein expression in the BNB. The chemokine-mediated and/or hyperproduction of interleukin-6 signalling has been implicated in the autoimmune neuropathies pathogenesis [168-170]. Clinically, GBS is characterized by limb or cranial-nerve weakness, loss of deep tendon reflexes, sensory, and dysautonomic symptoms due to peripheral nerves and root demyelination, and/or axonal damage. About 60% of all GBS are preceded by respiratory or gastrointestinal complications, with a presentation latency varying from 3 days to 6 weeks [170], which corresponds with COVID-19 GBS patients [171]. The suggested infection-mediated immune response that results in higher circulation of pro-inflammatory cytokines [171,170,59] reaches the peripheral nerve through the BNB.

Finally, the GBS and other peripheral nerve symptoms reveal that the peripheral nervous system (PNS) can be hijacked by SARS-CoV-2 through direct attack of microvessel endothelial cells, or indirect attack via immune mediated response. As ACE2 is widely expressed on the epithelial cells of the oral mucosa, SARS-CoV-2 can breach the BNB accessing the CNS via the cranial nerve using axonal transport machinery [172]. The upregulated vascular endothelial growth factor VEGF (C and A) is associated with COVID-19 endothelial barrier dysfunction [153,173], and specifically with the BNB [174]. As VEGF is also related to angiopoietins (Ang I and Ang II), accumulation of Ang II facilitates the elevation of VEGF and inversely augments Ang II, which forms a vicious cycle in the release of inflammatory cytokines including TNF-α, IL-1β, IL-6, IL-8, and ICAM-1, which causes BBB and BNSB disruption [173].

2.3. Blood-cerebrospinal-fluid-barrier (BCSFB)

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The choroid plexus (CP) of the BCSFB displays fundamentally different properties in comparison to the BBB. With a brisk blood flow (ten times higher than that of the brain) and highly permeable capillaries, the human CP provides the CNS with a high turnover rate of fluid (~400 mL/day) that contains peptides, micronutrients, and hormones for neuronal networks. BCSFB cells are the choroid plexus epithelium cells that line the cerebral ventricles and the arachnoid epithelium that line the brain vasculature in the subarachnoid space [112]. CP epithelial is considered to be less electrically resistant compared to the BBB endothelial, and is somewhat "leaky". Therefore, it is the prime target for viral entry into the CNS. Tight junctions (TJs) allow for endothelial cell occlusion and strict permeability by sealing off the intercellular space between the endothelial cells (ECs) lining the microvessel. TJs (e.g., occludins, claudins, and junctional adhesion molecules) are transmembrane proteins that bind intracellularly to the actin component of the filamentous cytoskeleton and extracellularly to identical transmembrane proteins in adjacent ECs [112].

As discussed under the BBB route, the SARS-CoV-2 particle could not be isolated and/or detected in the CSF, although the RT-PCR was positive and two fragments from ORF1a of the 1580-nucleotide were obtained [158,159], the CSF of those patients showed an albuminocytologic dissociation with increased protein level (98 mg/dL, reference value: 8–43 mg/dL) and normal cell count (2×10^6/L, reference value: 0–8×10^6/L) [171,175,176]. Eleven COVID-19–GBS patients from Wuhan, Italy, Spain, and France were analyzed. Although they all had very high concentrations of protein in CSF, in 7 of 11 tested patients, the virus was not detected in the CSF, suggesting that there is no direct root of intrathecal viral replication or infection. However, as intravenous immune globulin (IVIG) helped improve the condition of several patients, and one patient exhibited the presence of antibodies to the ganglioside GD1b, it appears that a post-viral-triggered immune response similar to other post-viral-induced GBS cases or other post-viral autoimmune neurologic disorders occurred [175]. As SARS-CoV-2 spike protein interacts with ganglioside dimers and the GalNAc residue of GM1 for anchoring to cell surface gangliosides, cross-reactivity between epitopes within the SARS-CoV-2 spike-bearing gangliosides and signature sugar residues of surface peripheral nerve glycolipids is very likely. Similar molecular mimicry has been shown between peripheral nerve glycolipids and Campylobacter jejuni or Zika virus [175]. This raised many questions, such as: how does this SARS-CoV-2 genomic material reach the CSF? Is it through the BCSFB? Is it accompanied by the delivery of the translated SARS-CoV-2 proteins? Are the increased CSF protein concentration and cell counts in COVID-19 linked with BCSFB and BNB permeability as immune-mediated responses?

3. Lymphatic brain drainage route

A third way SARS-CoV-2 can enter the CNS could be via the spreading of the virus in the lymphatic drainage system of the brain [177]. Although the glymphatic vessel structure mainly contains endothelial lining cell systems, this pathway is rather contradictory at the moment, and other researchers do not confirm lymphatic draining entry [2]. The lymphatic/glymphatic brain system (which is a glial-dependent waste clearance pathway in the brain that serves as a "front end" for the waste clearance connected downstream to an authentic lymphatic network) was discovered rather recently [178]. It has a vascular histological structure, which is quite similar to that of the endovascular blood system [179,180]. Importantly, endothelial lining cells of this system express both the *ACE2* and *TPMRSS2* genes, where the SARS-CoV-2 can access it. In line with these features, electron microscopy analysis revealed the presence of viral inclusion structures in endothelial cells [181]. The lymphatic/glymphatic system is different from traditional blood circulation since it is an open, afferent (one-way) system. The major

function of this system is to collect the soluble waste proteins and metabolic products from the CNS and drain them away. This unidirectionality raises an important and logical question, namely, how can it bring the viral particles to the CNS tissue, being a one-way drainage system? In addition, the lymphatic drainage function is impaired in age-dependent manner [179].

Therefore, the aforementioned BBB route is more favourable for CNS infection by SARS-CoV-2, especially in patients with severe COVID-19 complicated with the cytokine storm, which increases the BBB permeability, thereby facilitating the immune cell efflux into the affected tissues. At least in the severe cases, the virus (free or in vacuoles) is disseminated into many organs including the vasculature system, and can cause endothelilitis by attacking the endothelium. This may explain the frequently observed prothrombotic state with *in situ* clot formation and the impaired microcirculatory function across different organs in COVID-19 patients. Based on these observations it was suggested that one of the approaches to affect the course of COVID-19 would be to take some steps to stabilize the endothelium during viral replication, specifically in vulnerable patients with pre-existing endothelial dysfunctions, which are commonly associated with the male sex, smoking, hypertension, diabetes, obesity, and established cardiovascular disease, all being linked to adverse outcomes in COVID-19 [181].

4. Peripheral nerve or neuronal retrograde route: Accessing CNS via enteric, lungs, and kidney nerves routes

Although the direct neuroinvasion via hematogenous spread or migration of SARS-CoV-2 through the olfactory tract are possible infection routes to the CNS [150,3], the virus could also gain access from the periphery [182]. It has been postulated that brain stem invasion may occur via the vagal afferents from the upper airways, lung, and GI [40,182]. Furthermore, one cannot exclude the role of gastrointestinal (GI) tract involvement (a notion giving some tribute to the prospective gut-brain connection). In fact, the GI represents an important but mostly underestimated niche for SARS-CoV-2 replication. This is because the GI epithelium has higher relative expression of ACE-2 receptor than the lungs. Furthermore, SARS-CoV-2 can directly infect the intestinal cells and efficiently replicate there. Finally, it was pointed out that the clinical outcome were worse for COVID-19 patients with concomitant GI symptoms who required mechanical ventilation due to increased acute respiratory distress [183]. One study suggests that SARS-CoV-2 related-diarrhea and the GI dysfunction are not merely accessory symptoms, but serve as a possible marker of the involvement of the enteric nervous system/enteric glial cell (ENS/EGC) in pathogenesis, and suggests an alternative pathophysiological mechanism underlying SARS-CoV-2 neuroinvasion [182]. Here, the gut might serve as the “entrance door”, by which viruses may either directly neuroinvade or indirectly immunologically prime the enteric nervous system (ENS) or ascend to the CNS through intestinal vagal afferents [182]. This hypothesis is supported by the fact that there is a strict interconnection between the ENS and the enteric glial cells (EGCs) and that the gut epithelium is part of a neuro-epithelial unit crucial for gut homeostasis [182].

It was also established that in the case of MERS-CoV infection, the enteric involvement could take place before the respiratory infection [184]. Furthermore, brain infection was observed in mice infected with MERS-CoV either intranasally or by intragastric inoculation [184]. Although finding brain infection in mice with intranasal MERS-CoV injection is not surprising and was actuallyexpected, the fact that mice that underwent intragastric inoculation with MERS-CoV showed infectious virions in both brain and lung homogenates 5 days after the

inoculation was rather surprising [184]. The retrograde axonal transport and trans-synaptic transfer are well-documented for other types of coronavirus such as the avian bronchitis virus and the swine hemagglutinating encephalomyelitis virus (HEV) [166,167].

EGCs express the major histocompatibility complex class II and functionally work as antigen presenting cells for both innate and adoptive immune cells localized in the gut-associated lymphoid tissue (GALT) [185]. GALT houses many types of immune cells, such as T lymphocytes (γδ T lymphocytes). Upon activation by viral infection, GALT could initiate many immunological responses, such as transition of native $CD4^+$ lymphocytes into different subtypes (T_h1, T_h2, T_h17, T_{reg}, T_{sup}, and $CD8^+$). Furthermore, an enormous release of IL-6 and other inflammatory mediators also occurs upon activation, contributing to the acute respiratory distress as observed in the COVID-19-induced cytokine storm [63,182] and to the increase in the endothelium permeability, as aforementioned.

It was shown that the susceptibility to the SARS-CoV-2-inflicted GI damage of the inflammatory bowel disease (IBD) patients is determined by the dysregulated mucosal *ACE2* and *TMPRSS2* expression in the colon and ileum in IBD [186]. This deregulation was further enhanced by advanced age, smoking, and active disease that served as potential additional risk factors defining the vulnerability of IBD patients to COVID-19 through alterations of receptor expression [186].

Furthermore, it was established that both SARS-CoV and SARS-CoV-2 can efficiently infect enterocyte linage cells in human small intestinal organoids (hSIOs, which are the 3D structures that are grown from adult stem cells (ASCs) and recapitulate key aspects of the organ from which the ASCs were derived) [187]. This efficient infection of enterocytes in hSIOs by SARS-CoV and SARS-CoV-2 was demonstrated by confocal- and electron-microscopy, since the clusters of the extracellular viral particles (80-120 nm) were detected in the lumen of organoid and in the apical side of enterocytes associated with double membrane vesicles [187]. Could the viral particles of these disintegrated infected cells somehow reach and infect the glial intestinal cells, which are known to express ACE2 and TMPRSS2/furin? Could this scenario be repeated in other organs such as the heart, lungs, kidney, and even cutaneous tissues? Similar to SARS-CoV and SARS-CoV-2 infecting the human intestinal epithelial organoid [187], SARS-CoV-2 was shown to directly infect engineered human blood vessel organoids *in vitro* [188]. Using both immunochemistry and electron microscopy, SARS-CoV-2 viral particles were found in skin endothelium of patients presenting with chilblains (the painful inflammation of small blood vessels in the skin) [189]. As SARS-CoV-2 multiplies in the vascular cells of the skin area, can it go through the blood withdrawn by a mosquito bite? Ultrastructural examination identified typical CoV particles characterized by the spike structure in cytoplasm of hepatocytes in two COVID-19 cases [190]. Also, SARS-CoV-2 particles were found in heart, kidney, and lung autopsy of post-mortem samples [191]. Therefore, SARS-CoV-2 may first infect blood vessels' endothelial cells prior to infection of local tissues [188], and then be disseminated into many organs, including the human nervous system [188,181,189].

5. Somal cargo routes

5.1. Macrophage/monocytes cargo route

Some viruses are neurotropic, being able to invade nervous tissues and cause infections of immune-functioning macrophages, microglia, or astrocytes in the CNS [127,192]. Respiratory viruses may enter the CNS via a hematogenous or a neuronal retrograde route. In the first route, the virus disrupts the nasal epithelium and reaches the bloodstream and leukocytes, and - by

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manipulating the innate immune system - invades other tissues, including the CNS. Furthermore, in this route, leukocytes may act as a reservoir for viral transmission for neuro-invasive CoVs [64]. In the second route, the virus could infect peripheral neurons and access the CNS through retrograde trans-synaptic neuronal dissemination [64]. It is known that both alveolar and interstitial macrophages in the lungs express the ACE2 receptor and the TMPRSS2/Furin proteases, as well as ADAM-17, which acts as sheddase of ACE2. In the presence of all components of viral activation and binding, the virus can replicate in human macrophages [193] and dendritic cells [194], but the mature viral particles could not detected intra or extracellular from both of cells type. Furthermore, the electron microscopic post-mortem examination of the lung tissues clearly showed that the SARS-CoV particles and SARS-CoV-2 antigens are present and distributed in both alveolar macrophages, as well as in the lymph nodes and the spleen [195,196]. Previous data from 15 autopsies indicated that the SARS viral particles and genomic sequences were detected in a large number of circulating monocytes, lymphocytes, and lymphoid tissues. They were also found in the mucosa of the intestine, the epithelial cells of the respiratory tract, the neurons of the brain, the epithelium of the renal distal tubules, and macrophages in different organs, suggesting that the virus could infect multiple cell types in different organs [197]. One interesting find was that a large proportion of lymphocytes in the circulation and lymphoid organs contained the virus, as observed in the transmission electron microscopy (TEM) image of a circulating T lymphocyte in a patient who had SARS 6 days after onset of a fever, which may indicate that these circulating lymphocytes carrying viral molecules could reach the CNS and PNS via BBB, BCSFB, or BNB, or through all of these barriers. Based on this interesting result the mechanism of SARS pathogenesis was postulated [197], which appeared to be working until today. It is likely that the viral infection may convert these cells into long living macrophages (Mφ) and promote their migration into extra-pulmonary organ/tissues, where they become infected resident cells (viral reservoir) and as inflammatory signals producer, serve as a Trojan horse in other organs [193], with others cell types such as leukocytes, endothelial cells, smooth muscle cells, pericytes, inflammatory cells, neurones or glial cells [198]. A CD68 immunostain revealed that the macrophage infiltrated the cerebral hemispheres and subcortical white matter lesion associated with an axonal injury, and a perivascular acute disseminated encephalomyelitis (ADEM)-like appearance [198,65].

5.2. Double membrane vesicles cargo route

Many viruses have been shown to enter the extracellular double-membrane vesicle (EDMV) or exosome avenues during intra-host spreading and synthesis [199]. As previously reviewed in detail, all positive-sense single-stranded RNA viruses (including SARS-CoV and MERS-CoV) use, redirect, and rearrange host cell membranes as part of the viral genome transcription and replication tactic, harnessing their non-structural protein apparatus nsp1-16 [200]. This tactic allows them to produce double-membrane vesicles of different size and configuration, carrying different levels of viral particle structures, from dsRNA to full mature viral particles, which would be released as extracellular double-membrane vesicles (EDMV) or exosomes during the release from the host cells, or as a result of the post cell-host rapture. SARS-CoV-2 seems to be using a similar avenue of replication and release. This conclusion is based on the careful post-mortem histopathological electron microscopy analysis, and further confirmed using the *in vitro* SARS-CoV-2 cultured on Vero E6 cells [201]. In fact, electron microscopy revealed that the ultrastructural changes induced by both SARS-CoV and SARS-CoV-2 are

very similar and take place at comparable times after infection. However, the important differences between the two viruses were the facts that, 1) SARS-CoV-2 generated higher levels of intracellular viral RNA, but 50-fold less infectious viral progeny was recovered from the culture medium; 2) upon passaging in Vero E6 cells, SARS-CoV-2 was apparently under strong selection pressure to acquire adaptive mutations in its spike protein gene [201]. These mutations changed or deleted a putative furin-like cleavage site in the region connecting the S1 and S2 domains of the S-protein and resulted in a very prominent phenotypic change in plaque assays [201].

Of note, SARS-CoV-2 could infect almost all human body organs and tissues [189,153,200]. The infected cells shedding the exosomes (EDMVs). Furthermore, apoptotic or the diffuse damage of infected cells can lead to the release of their contents in a form of different types of EDMVs containing different viral structures (ranging from viral dsRNA to mature viral particles), to infected the adjacent new cells/tissues and expanded to circulate systemically and disseminate to reaching distant tissues [200]. This hypothesis is supported by [102,189,155], where viral particles were detected in endothelial cells of lungs, kidneys, skin chilblains, and central nervous system of vascular system and organs-crosstalk via the vascular system. The transmission electron microscopy analysis of the post-mortem frontal lobe brain sections showed the presence of viral particles. Pleomorphic spherical viral-like particles having variation in the size and shape were found either individually or within the small vesicles of endothelial cells. The possible active pathogen transcellular penetration (entry-transit process) across the brain microvascular endothelial cells into the neural niche was evidenced as blebbing of viral-like particles coming in/out of the endothelial cells wall. Neural cell bodies exhibited distended cytoplasmic vacuoles containing enveloped viral particle exhibiting electron dense centers with distinct envelope projections ending in round 'peplomeric' structures typical of a coronavirus particle [102]. In the light of these findings, it seems logical that the virus can access the brain directly through the permanent destabilization of the BBB, specifically in patients with very severe viral infection complications accompanied by systemic inflammatory responses, in its free form or vacuolated in double membrane vesicles. In turn, not only viral particles but the peripheral cytokines can gain entry to the CNS, and consequently exacerbate or trigger neuroinflammation that can result in many neurological manifestations, including encephalitis [34].

The isolated EVs released from DENV-2 infected U937 macrophage cell line carrying the viral NS3 protein and different miRs induced an increase in the polarization of the endothelial (EA.hy926) monolayer cells permeability, as well as changes in the expression of ICAM and the VE-cadherin , also leading to an increase in the levels of the IP-10, TNF-α, RANTES, IL-10, and MCP-1 secretion, even in the absence of the virus [202], suggesting that a proinflammatory status was involved in the endothelial permeability alteration. The miRs most frequently counted within the vesicles obtained from such DENV infected cells include the miR 21, miR 92a, and miR 191, which are strongly associated with many biological pathways involving endothelial cell processes, such as tubular network formation, angiogenesis, and brain microvascular reparation. Such vesicles obtained from the DENV infected cells induced an endothelial activation, possibly determined by the miR that they contain [202].

Different cultured glial cell types released the EDMVs, as well as EDMVs of different sizes were detected in CSF. EDMVs are able to cross the BBB in both directions, though it is unclear what the route of transfer is. Also, the peripheral EDMVs can interact with the BBB leading to changes in the barrier's properties. EDMVs can enter the brain parenchyma at the choroid plexus and to facilitate folate import into the brain. Of note, the inflammatory conditions often associated with a leaky BBB facilitated the entry of peripheral EDMVs into the brain, resulting

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in genetic modulation of the target cells of the CNS. These results indicate to that the EDMVs may act as a means of the non-synaptic neuronal cell communication, hence the EDMVs released from neurons are likely involved in the transfer of biomolecules across synapses. Furthermore, all types of macroglia and microglia (phagocytic cells) contributing to CNS tissue homeostasis can secrete EDMVs in the form of exosomes or microvesicles. Microglia (reviewed in [115,203,204]).

6. COVID-19 brain access via nicotinic acetylcholine receptor (nAChR)

It is known that there are functional connections between the ACE2 expression and nicotine exposure in lungs and other organ systems, such as the kidneys and heart. It is likely that because of these functional connections and due to the capability of nicotine to interact with the components of other renin angiotensin system (RAS), smoking can promote COVID-19 cellular entry through the nicotinic acetylcholine receptor (nAChR) signalling pathway. Notably, nAChRs are found on the surfaces of many of the same cells that express ACE2 in the kidneys, lungs, circulation, and in the brain and immune cells [205-208]. Therefore, smoking can impact COVID-19 pathophysiology and have a clinical outcome in several organ systems [209]. As ACE2 is expressed in the brain and functionally interacts with nAChRs [210-212], it was hypothesized that if neural cells, such as epithelial cells, are more vulnerable to infection in smokers since nicotine stimulation of the nAChR can increase ACE2 expression within them [213]. This is an important point since it was shown that mRNA from the closely related SARS-CoV, which also binds ACE2 as a mechanism of cell entry, can be detected in brain and cerebrospinal fluid of infected individuals [214-216]. Furthermore, SARS-CoV's ability to enter neurons was established in the experimental systems using recombinant human ACE2 as the point of entry [44,217]. Considering this scenario, Olds and Kabbani [213,209] asked important questions, such as: can the COVID-19 infection lead to long-term neural damage in both symptomatic and asymptomatic individuals? And if it can, then can the chronic nicotine exposure through smoking habits and addiction increase the risk of the developing of COVID-19-associated neuropathology through interactions between nAChRs and ACE2 in neurons and glia? These important questions still wait for their answers.

7. Immune-mediated responses and SARS-CoV-2 neurological complications

Figure 3 represents three escalating phases of COVID-19 disease progression. Increased vascular permeability is also a hallmark change that occurs in the process of a cytokine storm [218]. Although it is known that the cytokine storm (hypercytokinemia) has devastating effects on the respiratory system promoting hyperinflammation and ARDS, and serves as one of the major causes of the fatal outcomes of the disease, the neurological effects of the cytokine storm are less understood. For example, it is not clear if acute or subacute CNS involvement can be caused by the cytokine storm occurring during the final stage of the disease. A case was reported, where a COVID-19 patient developed acute necrotising haemorrhagic encephalopathy after several days with altered mental status, cough, and fever [52]. Although at this stage, the patient demonstrated BBB disruption and the intracranial cytokine storm, no direct viral invasion of the CNS was observed in this case [52].

Importantly, the nervous system seems to be affected by the alterations in the neuroinflammatory mechanisms. In fact, immune mechanisms similar to those initiating the

cytokine storm in SARS are known to be related to the pathogenesis of multiple neurological diseases, such as cerebrovascular disease, peripheral nervous system disorders, and postinfectious immune-mediated encephalitis, and [219]. Furthermore, a proinflammatory environment is known to play a role in the pathogenesis and progression of a wide range of neurodegenerative diseases, such as AD, amyotrophic lateral sclerosis (ALS), Huntington disease, multiple sclerosis (MS), and PD, where a chronic neuroinflammation causes high levels of cytokines/chemokines [219]. Many of these neurodegenerative diseases are age-related, and the efficiency of the innate immune response is decreased in older age, increasing the vulnerability of these patients to infection [220]. Older individuals also demonstrate greater severity of the immune response against SARS-CoV-2 infection. All this indicates that there is a potential association of the development and progression of neurodegenerative diseases with SARS-CoV-2 infection that requires careful analysis and better understanding.

Furthermore, although children and adolescents typically demonstrate rather mild COVID-19 course, one cannot exclude the possibility that the SARS-CoV-2 infection may have prospective long term neurological consequences in these population groups as well, triggering some cognitive and psychiatric disorders. In fact, synaptic pruning during childhood and adolescence can be distorted by the immunological alterations associated with SARS-CoV-2 infection, causing problems that will only become apparent in adulthood [219].

Both, adaptive and innate immune responses against SARS-CoV-2 infection and virus itself may cause damage within the CNS or PNS. In fact, both endothelial cells of the BBB and epithelial cells of the BCSFB in the choroid plexus (CP) located in the ventricles of the brain can be targeted by the virus. Furthermore, similar to other viruses [221] SARS-CoV-2 can use leukocytes as an intermediate host cell before spreading into CNS from circulatory system [222]. In fact, such “Trojan horse” mechanism [223], where circulating leukocytes are used by viruses to carry them across the BBB, was described for HIV [224], Zika virus [225], and HCoV-229E [226-228]. In other words, SARS-CoV-2 is potentially able to establish a reservoir in leukocytes converting them into the delivery vehicles disseminating infection outside the respiratory tracts and spreading it into the other tissues including CNS [222].

Transmission electronic microscopy analysis of a brain tissue specimen obtained from the SARS patient succumbed to encephalopathy revealed the presence of SARS-CoV-like viral particle, and a SARS-CoV strain was isolated from this sample, clearly demonstrating the neurotropic potential of this virus [229]. Furthermore, cytokine/chemokine assay showed elevated expression of a cytokine, monokine induced by interferon-γ (MIG), and interferon-γ–inducible protein 10 in this sample, and the immunohistochemical analysis revealed that a major source for MIG production in the brain was gliocytes [229]. These findings supported the idea that viral entry to CNS might trigger the infiltration of immune cells and the release of cytokines and chemokines, which contribute to the BBB permeability and/or damage.

Therefore, there is clearly an important interplay between the SARS-CoV-2 and the immune system, where dysfunctional immune responses contributes to the disease progression [230]. For example, rapid viral replication and secondary cellular injury during the SARS-CoV-2 promoted the increase in the secretion of inflammatory cytokines, such as IL-4, IL-10, IFN-γ, IL-1β, and TNF-α, and the cytokine storm is initiated when the levels of released cytokines are injurious to host cells. The presence of a cytokine storm in severe COVID-19 patients is suggested by their high plasma levels of the inflammatory cytokines [231]. The presence of a cytokine storm combined with the elevated D-dimer (which is a fibrin degradation fragment produced when a blood clot gets dissolved in the body, and which, therefore, serves as a reflection of the presence of thrombosis (blood clotting) and/or thrombotic embolism) and ferritins levels were also reported in SARS-CoV-2 infected patients [232,63].

Previous studies on sepsis revealed that sepsis-associated cognitive impairment and other neurological symptoms can be triggered by the cytokine storm-induced BBB disruption and resulting neuroinflammation [233]. This clearly indicates that the activation of the immune system is not only protecting the organism, but also is capable of inducing serious harm, thereby representing a double-edged sword. Contributing factors to the harmful side are the overactivation of the immune system, infection-induced cytokine storm, and the increased immunoglobulin levels in CSF [234]. In line with these mechanisms, neurologic features in severe SARS-CoV-2 infection were shown to be combined with the elevated IgG levels and the presence of the oligoclonal bands (which are defined as at least two CSF electrophoresis bands seen in the CSF samples with no corresponding band present in the serum) in the CSF [234]. Furthermore, the development of acute necrotizing encephalopathy or Guillain–Barré syndrome in virus-infected patients can be associated with intracranial cytokine storms leading to the breakdown of the BBB without direct viral invasion of the CNS [175]. In the classic view, acute lung injury (ALI) and acute respiratory distress syndrome (ARDS) represent an inflammatory disruption of the epithelial and endothelial cellular barriers of the alveolar-capillary unit, with ensuing microvascular hyperpermeability and flooding of alveolar spaces [235].

Another illustration of the double-edged sword concept of immune response activation in SARS-CoV-2 infection is given by the complement system, activation of which represents the first response of the host immune system against SARS-CoV-2 infection. However, “everything is good, which is good in moderation”, and uncontrolled complement activation can be harmful as well. In fact, the virus infection of the lungs and other organs can cause complement over-activation leading not only to the acute and chronic inflammation, but to the vasculopathy, e.g., endothelial cell dysfunction, intravascular coagulation, and thrombus formation, thereby contributing to the multiple organ failure and death [236]. In other words, such uncontrolled complement activation might initiate some terminal pathways accounting for what clinicians and pathologists are observing in COVID-19 patients, that is, “although the lungs are ground zero, the virus’ reach can extend to many organs, including the heart and blood vessels, kidneys, gut and brain” [236,237]. The activation of complement component C3 exacerbates SARS-CoV–associated ARDS [238], whereas C3-C5 complement deposits are abundant in the lung biopsies from patients with COVID-19 [239]. C5a signalling through its G-protein coupled receptor C5aR1/CD88 increased BBB permeability in neuroinflammatory disease settings *in vivo* [240]. It is highly likely that “inflamm-aging” (which is a chronic progressive increase in the proinflammatory status associated with the aging process [241]) is correlated with increased risk of a cytokine storm in some critical elderly patients with COVID-19 infection [242].

Conclusions

There are numerous pathways that can be utilized by SARS-CoV-2 to breach the body’s defences reach the peripheral nervous system (PNS) and central nervous system (CNS). SARS-CoV-2 or its components reach the nervous system through direct contact specifically in severe COVID-19 cases, or indirect contact through multiple mechanisms of immune-mediated responses in mild to moderate COVID-19 cases. We are discussing here that there are at least seven candidate routes, which the mature or immature SARS-CoV-2 components could use to reach the CNS and PNS, utilizing the within-body crosstalk between organs. Obviously, utilization of any one of these routes is sufficient to make SARS-CoV-2 neurotropic.

Significance Statement

There are neurological manifestations and complications of the SARS-CoV-2 infection. Therefore, similar to other coronaviruses SARS-CoV-2 is a neurotropic virus. To answer the question on how SARS-CoV-2 infection can reach the human nervous system, we are discussing here seven candidate routes. Among these seven pathways, the blood-endothelial-barrier is the main route for SARS-CoV-2 entry into the nervous system. An important other route is breaching of the BBB, permeability of which can be increased by the cytokine storm leading to neuroinflammation.

Data Availability Statement

Data sharing not applicable to this article as no datasets were generated or analysed during the current study

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Figure legends

Figure 1: Respiratory and extra respiratory organ/system COVID-19 prevalence.

Figure 2: Diagram for human blood-nervous-system-barriers. **A**. Blood-Cerebrospinal Fluid (BCSFB)/ choroid plexus barrier (1. Fenestrated endothelium, 2. Interstitial matrix, 3 and 4. Choroid plexus epithelium, 5. Brain cells). **B**. Blood-Cerebrospinal Fluid (BCSFB)/Meningeal barrier (1. arachnoid, 2. Trabeculae cross section, 3. Pericyte, 4. Epithelial cell tight junction, 5. Epithelial cell, 6. CSF, 7. Pia Mater). **C.** Blood-Brain Barrier (1. Astrocyte, 2. Basement membrane, 3. Pericyte, 4. Endothelial cell [non- fenestrated], 5. Tight junction). **D1**. Blood Nerve barrier BNB (cross section) (1. Epineurium, 2. Perineurium, 3. Endoneurial vessel, 4. Basal lamina. 5. Endoneurium, 6. Myelin, 7. Nucleus of Schwann cell, 8. Axon, 9. Endoneurial endothelial cells of microvessel, 10. Epineurium blood vessel). **D2**. Blood-nerve Barrier. **E**. Blood olfactory nerve barrier. **F**. Inside the glomeruli barriers both of glomerular endothelial and epithelial cells (known as podocytes) crosstalk occurs, where they share the glomerular basement membrane. **G**. inside the glomerular both of peritubular capillary endothelial and tubular epithelial cells crosstalk also occurs through the barrier where they are separated by a tubular basement membrane and interstitial space. Both of kidney epithelial (podocytes and tubular) are breached with SARS-CoV-2 in COVID-19 patients (reviewed in [200]). From her the viral and/or its components can spread from renal anastomosis into CNS via nerve supply. Although the crosstalk between human organs in health and diseases is a very complicated processes go through huge number of mechanisms, but it well documented in a dramatically pattern [243,244].

Figure 3: Classification of COVID-19 disease stages. The figure illustrates three escalating phases of COVID-19 disease progression, with hypothesis of blood-endothelial/epithelial barriers integrity/permeability scale associated with age and comorbidities diseases over the three stages. The blood-endothelial barriers are representative for all body barriers and specifically for blood-Nervous system Barrier (BNSB). Progressive increase in inflammatory cytokine and chemokines eventually leads to cytokine storm in a profile similar to in sepsis cases, which eventually leads to endothelial-barriers dysfunction. Many other biomarkers molecules (in addition to the cytokine storm elements) have a direct effect on the BNSB as discussed in text. The times on the X-axis are approximate. The figure designed based on and adapted from [200,245,246,114,113,247]. + to >4+ indicative for barrier integrity/permeability like scale, IIA (stage II without hypoxia) IIB (Stage II with hypoxia). Tumor Necrosis Factor (TNF-α), Interleukin 1β (IL-1β), IL-6, GCSF: granulocyte-colony stimulating factor, interferon gamma-induced protein-10, monocyte chemoattractant protein-1, and MCA-protein 1: macrophage inflammatory proteins 1-α.

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Table 1: Ultrastructure features of SARS-CoV-2 viral particles in human nervous tissues and endothelial system

Ref.	Sample type	Sample source	Viral particle size	Virus features
[102]	post-mortem	frontal lobe tissue	80-110 nm	two morphologically distinct types of spike protein structures, typical of betacoronaviruses, viral particles in frontal lobe brain sections, Individual and in small vesicles of endothelial cells of a Pleomorphic spherical viral-like particles were observed, Blebbing of viral-like particles coming in/out of the endothelial wall which pointing to presumed active pathogen entry-transit (transcellular penetration) across the brain microvascular endothelial cells into the neural niche was recorded, Neural cell bodies exhibited distended cytoplasmic vacuoles containing enveloped viral particle exhibiting electron dense centers with distinct stalk-like peplomeric projections
[155]	Autopsy within three hours post-mortem	Human .Olfactory nerve, . Gyrus, . Brainstem	98-160 nm	Spherical particle with crown-like shape and inner dense core and electron-dense periphery, double nuclear envelope, severe damage in the olfactory nerve, autophagy phenomena appeared in the cytoplasm
[229]	post-mortem autopsy	Autopsy brain tissue was cultured with Vero E6 for E.M.	~80–90nm	Clear cytopathic effect, enveloped virus particles with morphology compatible with coronavirus., Extracellular particles were found clustering and adhering to the surface of the plasma Membrane, the Immunostaining demonstrated that monokine induced by interferon-γ (Mig) expressed in gliocytes with the infiltration of CD68+ monocytes/macrophages and CD3+ T lymphocytes in the brain mesenchyme
[181]	post-mortem autopsy	Human transplanted kidney	150 nm	Viral inclusion bodies in peipenilubular space and viral particles in endothelial cells, aggregates of viral particles with dense circular surface and lucid centre, capillaries containing viral particles.
[189]	skin biopsies	Human chilblains	92.26 nm	Immunohistochemistry and transmission electron microscopy presented the viral particles within endothelial cells in lesion skin biopsies from patients presenting with chilblains. Ultrastructural examination revealed the presence of round membrane-bound structures within the cytoplasm of endothelial cells showing an electro-lucent centre, and surrounded by tiny spikes, giving them a halo-like appearance. Their mean diameter was 92.26 nm (80.76-109.76 nm), and the mean thickness of the spikes was 13.18 nm (12.36-13.88 nm)
[153]	Pulmonary autopsy	Human pulmonary	-	SARS-CoV-2 particles within the destructed lung vascular endothelial cell, which expressed 8.3-fold more ACE2 than non COVID-19 samples. A total of 79 inflammation-related genes were differentially regulated only in specimens from patients with Covid-19.

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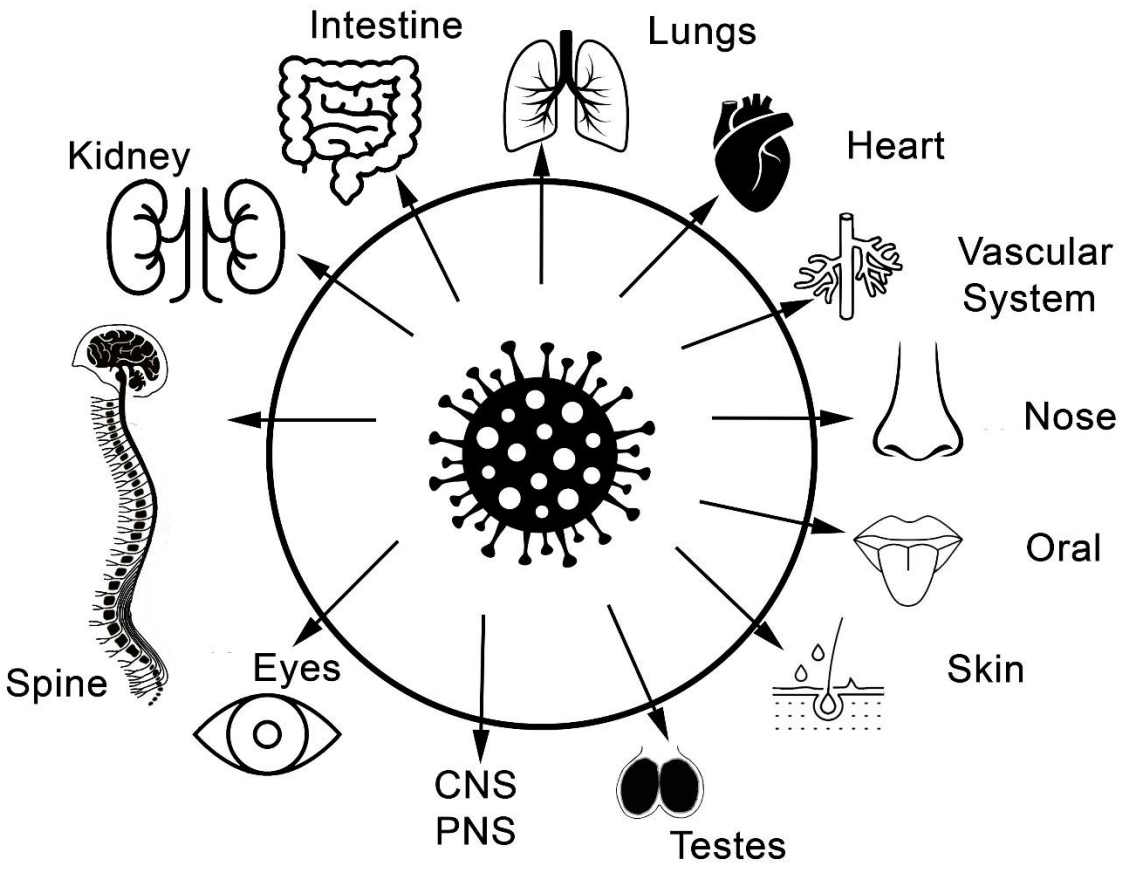

Figure 1

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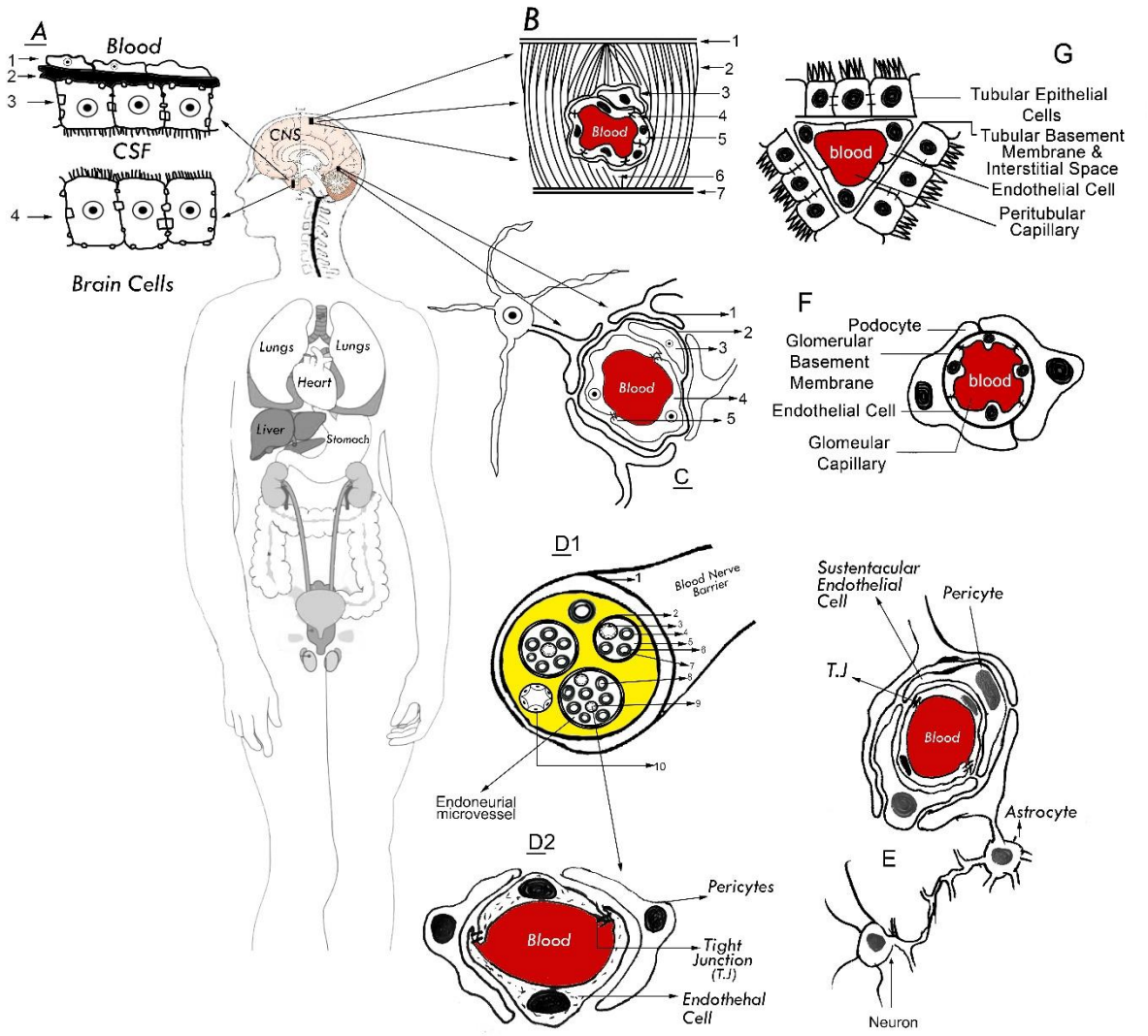

Figure 2

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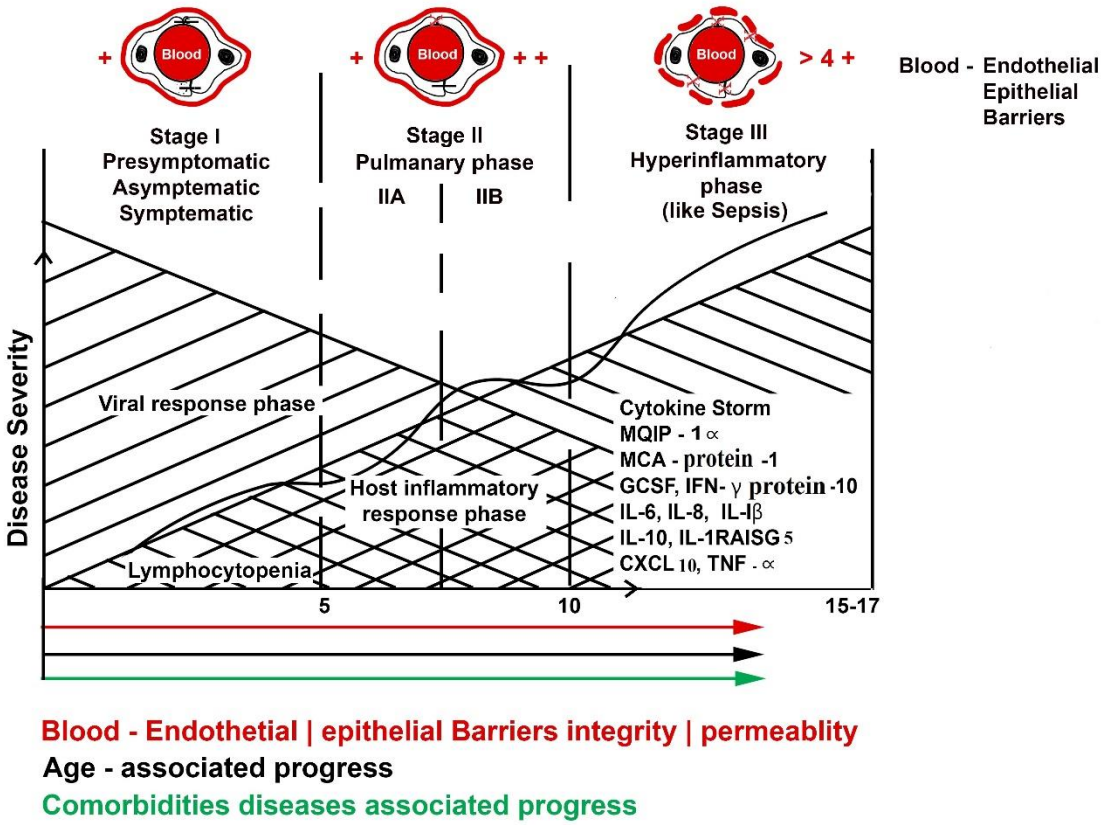

Figure 3