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Chapter

Neurotropic SARS-CoV-2: Causalities and Realities

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Abstract

Evidences for the dysfunctions of central nervous system (CNS) caused by SARS-CoV-2 infection have accumulated since the beginning of pandemic. The clinical and experimental evidences on viral entry routes to CNS lead to several open questions. While the neurological impairments caused by the virus stay as a reality under Long COVID, dissecting the causality underlying these problems continues to be an intensely studied topic. Extensive reports of olfactory dysfunctions including anosmia, hyposmia, and parosmia due to infections during 2020–2021, led to the hypothesis of virus' CNS invasion through the olfactory nerve. Some of the investigations using animal models of cellular factors mediating the viral entry also suggest potential neurotropism. Conversely, recent studies proved the absence of viral particles in olfactory sensory neurons and olfactory bulb, hence leading to the deliberation on viral entry route. Here, we summarize the findings on the debated neurotropic characteristics of the virus, including clinical observations and the results from animal models. Further, we emphasize on the need of tracking olfactory and cognitive fitness in the post-COVID-19 era.

Keywords: SARS-CoV-2, olfactory fitness, neurotropism, cognitive impairments, neuro-COVID

1. Introduction

The Coronavirus disease 2019 (COVID-19) pandemic poses an unparalleled challenge to the public health in dealing with long-term adverse effects of the infection. Several neurological complications have been reported to be associated with COVID-19. At the beginning of pandemic, in one of the early correspondences on autopsy studies published in the New England Journal of Medicine reported the presence of SARS-CoV-2 in multiple organs including the brain [1]. Recently published brain imaging data from subjects who were scanned before and after infection show structural abnormalities in the central nervous system (CNS). Significant changes were found in the brain areas that are functionally connected to the primary olfactory cortex, orbitofrontal cortex, and olfactory tubercle. This suggests possible long-term cognitive impairments due to COVID-19 infection in the central nervous system (CNS) that may happen through olfactory mucosa [2]. These findings support early reports on the presence of SARS-CoV-2 RNA and protein in the nasopharynx [3]. However, the postmortem studies of olfactory and respiratory mucosa confirmed

sustentacular and ciliated cells as the targets for SARS-CoV-2 infection. There were no evidences found in this study for the presence of viral particles in the olfactory sensory neurons (OSNs) or olfactory bulb (OB), questioning the neurotropism shown by the virus [4]. These contrasting results prompt us to carry out a narrative literature review on the reported causalities and realities on the neurotropic characteristics of SARS-CoV-2.

One of the virus entry routes, i.e., binding of viral spike (S) protein to the human angiotensin-converting enzyme 2 (hACE2) receptor and the S protein priming by host cell transmembrane protease, serine 2 (TMPRSS2) was uncovered at the beginning of pandemic [5]. These cellular factors are present in the non-neuronal cells of human olfactory epithelium, cortical neurons, Purkinje neurons, cerebellar and cortical astrocytes, etc. [6, 7]. Another receptor type that can mediate the infection, Neuropilin-1 (NRP1), is abundantly found in the neurons, olfactory epithelial cells, and endothelial cells [8, 9]. Other potential route can be through the ACE2 receptors present on the endothelial cells, thereby using the vascular system to attack the blood-brain barrier and to get access to the CNS [10]. Thus, despite the entry route to CNS being a debated topic, these evidences can be used to explain the pathophysiology of neurological impairments and long-term cognitive dysfunctions caused by COVID-19 infection. In this chapter, we are summarizing the evidences for the debated topic of SARS-CoV-2 neurotropism, the importance of quantifying olfactory and cognitive fitness in the context of Neuro-COVID and the studies in model systems that suggest neurotropism. To this end, we have carried out the literature review using a combination of keywords such as "SARS CoV2 entry routes to brain" and "Olfactory and cognitive impairments due to COVID-19" and "animal models of CoV-2." We have mostly used Google Scholar and PubMed to search for the articles. As we are aiming to provide a narrative overview on the debated topic of Neurotropic SARS-CoV-2, we are summarizing only the selected and relevant findings on this topic.

2. Entry routes of SARS-CoV-2

To investigate the pathophysiology associated with SARS-CoV-2 infection, one of the critical steps is to mechanistically discern the routes of its entry into the host. Unprecedented research is underway, since the beginning of the COVID-19-induced pandemic to tease out the different entry points of the novel SARS-CoV-2 in the human body. It has been confirmed that CoV-2 virus presents the spike glycoprotein to the cell membrane for binding to the human angiotensin-converting enzyme 2 (hACE2) receptor [11, 12]. It is famously referred to as the SARS-CoV functional receptor [13]. One of the imperative functions of hACE2 protein is maintaining the neural homeostasis by regulating the renin-angiotensin signaling (RAS) system [14]. A seamless entry into the cell is warranted by the cleavage of S2' site of the virus by the TMPRSS2 after engaging with hACE2 at the membrane [5, 15, 16]. In the endosomal compartments of the cell, the cleavage is mediated by Cathepsin L protease, which initiates formation of the fusion pores [17, 18]. Inside Golgi apparatus, Furin protease cleaves the virus into S1 and S2 compartments [19]. After successful entry and proteolytic cleavage, viral machinery is assembled and activated to spread the infection [16].

Importantly, the agents that allow SARS-CoV-2 entry, specifically, human-ACE2 (hACE2) are present across different bodily tissues including the brain [14]. Such a widespread expression in the body would allow for conjecturing several routes by which virus can enter and invade. Indeed, the repertoire of symptoms associated with

COVID-19 is a testimony to the tropism of virus in different cell types and tissues. Studies involving bulk and single-cell RNA sequencing revealed ACE2-TMPRSS2 expression in the different cell-types such as the sustentacular (SUS) cells, respiratory ciliated and secretory cells as well as the horizontal basal cells of the respiratory and olfactory epithelium (RE and OE) of human nasal mucosa [20, 21]. Other peripheral routes include that of the eye and oral tissues [22, 23]. Virus specimen was found to be present in the conjunctivital and tear swab of patients [24, 25]. Indeed, the viral entry machinery components, ACE2 and TMPRSS2, are present in conjunctivital epithelium and the epithelial and endothelial parts of the cornea [26]. Oral cavity also allows viral entry due to the enrichment of the entry proteins in the epithelial cells of the salivary glands and mucosae found in the single-cell RNA sequencing data of human samples [27]. Entry via oral route suggests correlation of salivary viral titer with the taste loss observed in COVID-19 patients [27, 28].

CoV-2 virus can potentially breach the blood-brain barrier (BBB) as a result of the barrier instability caused due to the increased number of inflammatory cytokines upon infection [10, 29]. Viral invasion of the brain areas by gaining entry from the circumventricular organs (CVOs) and brainstem structures could also serve as plausible routes in the patients who suffer from massive cytokine storm or those having compromised health prior to the infection [30]. One of the cytokines, tumor necrosis factor-α (TNF-α) can enter the BBB or in CVOs (structures lining the ventricles with accessible vasculature), which can activate downstream microglia and astrocytes [31]. The activated cells, in turn, can cause damage to the neurons via excitotoxicity and thereby impair the signaling processes of the brain [30]. Fecal-oral routes are yet another proposed route of viral dissemination in the body [32]. It is, however, not confirmed that this transmission route is responsible for gastrointestinal symptoms associated with COVID-19. It has been hypothesized that movement via vagal and spinal axonal fibers can allow viral invasion of the GI tract. Occurrence of syncope in patients with normal electrocardiogram assessment hints toward changes in the neural control of blood pressure changes [33, 34]. In one study, patients with syncope indeed had a significantly lower increase in the compensatory heart rate compared with those non-syncope ones, which suggested plausible impairment in the baro-reflexive control. Such an acute hypocapnic hypoxemia could have occurred due to CoV-2-mediated ACE2 internalization in specific midbrain and medullary nuclei, which can lead to impairments in baroreflex and chemoreceptor responses [33]. Malfunctioning of brain-lung axis can be indicated as severe lung and chest CT abnormalities and defects observed in neuroimaging analysis. The sensory neurons lining the airways can sense the virus induced inflammatory responses in the lungs and provide feedback to the brain [35, 36]. The carotid body sinus nerves innervating this organ can profoundly play a role in the retrograde transport of the virus to the brain. Carotid body invasion by virus due to local expression of ACE2 can cause impaired peripheral arterial chemoreception leading to hypoxic and hypercapnic conditions with changes in pH [37]. Separately, the mucosal immune system, which comprises the lymphoid tissues of the gut and the lungs, provides the clue for dissociating the components of gut-lung axis in mediating dysfunctions associated with the COVID-19 infection. Translocation of the active immune cells from gut to lungs can exacerbate inflammation, even causing lung injuries and respiratory distress. Within the gut, CoV-2 can downregulate ACE2 expression causing microbial dysbiosis and further affecting lungs via the gut-lung axis. Finally, neural control of the cardiovascular processes under COVID-19 infectious condition is yet another axis via which the virus can act and affect these organ systems [38]. Cardiac arrhythmias in COVID-19

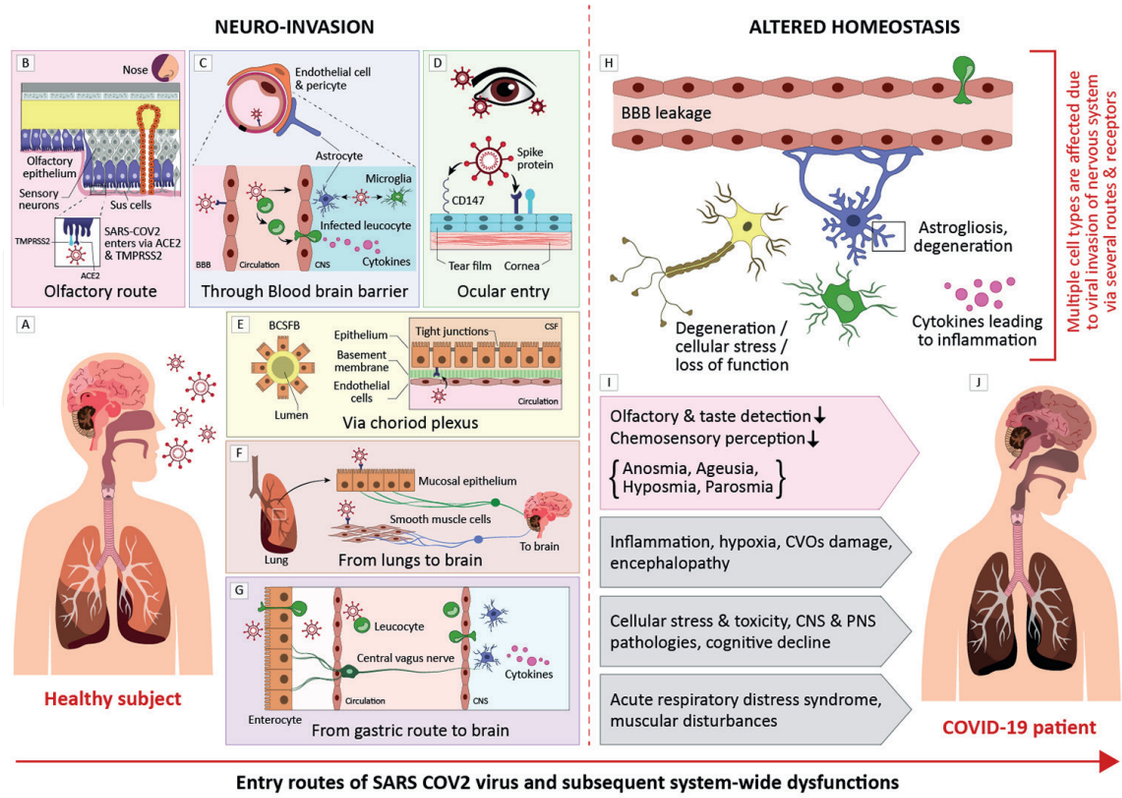

Figure 1.
Neuroinvasion by SARS-CoV-2 and subsequent dysfunctions. (A) Infection of a healthy subject by SARS-CoV-2 leads the entry of virus into different organ systems. (B–G) Routes and cell-types through which the virus can enter and invade the nervous system, i.e., via, olfactory route, via blood-brain barrier, eye, choroid plexus, blood-cerebrospinal fluid (CSF) barrier, via lungs to the brain and through the gastric enterocytes to the central nervous system [7, 40–42]. (H) Altered homeostasis can occur as a result of neuroinvasion by CoV-2 virus leading to detrimental effects at multiple cell types of the nervous system, i.e., neuron (yellow), astrocyte (blue) and microglia (green) [43]. (I) System-wide dysfunctions ranging from cellular to olfactory to CNS and PNS pathologies have been reported in COVID-19 patients. (J) An infected COVID-19 patient with different bodily systems affected due to the viral tropism.

patients are mostly occurring due to direct myocardial damage by CoV-2 infection or via the systemic inflammatory responses [39]. Arrythmias can also indirectly be caused by dysfunctional neural control of the heart rate. There are feedback mechanisms to the brain for maintenance of the cardiac rhythm and for dampening the production of cytokines and other inflammatory mediators in case of infection [38]. It could be that the severe CoV-2 infection can alter the neural feedback mechanisms of cardiovascular control. Apart from the olfactory route, these three axes i.e., the lung-brain, gut-lung, and heart-brain may also serve as the routes of transmission and invasion by the virus leading to multiple organs dysfunctions and manifestation of a variety of symptoms and conditions (**Figure 1**).

3. SARS-CoV-2: neurotropic or not?

The CNS is an immune privileged system of the body, owing to the highly protective brain-cerebrospinal fluid barrier, blood-brain barrier as well as surveillance by innate immune sentinels [44, 45]. Viral adaptations can allow multiple entry routes, either via the peripheral nerves or through the hematogenous routes. This can lead to neural and endothelial destruction causing CNS dysfunctions [46]. A variety of

neuropathological viruses are the respiratory viruses belonging to the categories of the influenza virus (IV), the coronaviruses (CoV), human metapneumovirus (hMPV), and human respiratory syncytial virus (hRSV) [47–49]. They are known to enter the CNS through various routes and mechanisms and invade the system leading to long-term neurological sequalae in the patients [49]. Such viral infections, under severe conditions, usually lead to neurological impairments such as encephalitis, seizures, epilepsy, and other encephalopathies [50–52]. In subsequent section of the chapter, we review the neuroinvasive nature of CoVs and how studying them over the years has helped us understand the SARS CoV-2-mediated complications better. We will also highlight the ongoing debate about the neurotropic nature of the SARS-CoV-2 in the upcoming subsections.

3.1 Neuro-invasive capabilities of coronaviruses

The three kinds of epidemic-causing CoVs, the SARS CoV-1, Middle East Respiratory Syndrome (MERS) CoV, and the currently prevailing SARS CoV-2, have all been demonstrated to exhibit neurological invasive capabilities [53, 54]. Viral encephalitis, i.e., lesions in the brain parenchyma including neuronal damage caused due to virus, has been confirmed in COVID-19 patients [55]. Genomic sequencing of viral particles in the CSF of the patient verified the case of encephalitis [56]. In fact, encephalitis, polyneuropathy, and aortic ischemic stroke were also commonly observed in severe cases of SARS-CoV epidemic that occurred in 2003 [53]. SARS CoV-2 shares 79.5% genetic similarity with SARS-CoV virus, and hence, finding out the mechanisms of neurological impairments by CoV-2 might be more tractable [57]. Acute viral infection causing hypoxia, systemic toxemia, and other metabolic disorders can result in toxic encephalopathy. It is mainly characterized by cerebral edema and symptoms include headache, delirium, dysphoria, and in extreme cases, lead to loss of consciousness, coma, and paralysis [58]. COVID-19 patients often suffer from hypoxia, viremia, and even headache and disturbed consciousness, which can potentially lead to acute toxic encephalopathy. In extreme cases of COVID-19, enhanced cytokine storm, increased levels of D-dimer, and reduced platelet count can even allow viral-induced cerebrovascular events to occur [59]. Multiple reports of confirmed viral infection in the brain have been narrated since the beginning of the pandemic outbreak. MRI scan of a young COVID-19 female patient with mild symptoms and normal chest CT showed significant cortical hyperintensity in the right gyrus rectus and subtle hyperintensity in the OBs suggestive of viral invasion in these brain regions [60]. In autopsies assessment of brains of six patients who suffered from COVID-19, brainstem neural damage, meningitis, and pan-encephalitis were reported [61, 62]. HCoV-OC43, HCoV-229E, and SARS-CoV-1 are the human-infecting CoVs, which are capable of infecting the neurons directly, apart from causing CNS damage due to immunological and inflammatory responses [63, 64]. HCoV-OC43 has been associated with multiple sclerosis (MS) with its RNA detected in the CSF of 12 of 22 patients suffering with MS [65]. MERS-CoV, although, enters via a dipeptidyl peptidase receptor and has affected only ~2500 individuals since 2012, it also has been shown to generate neurological impairments such as seizures, headaches, and perceiving confusion [66]. Cases of Guillain-Barrè syndrome (GBS), axonal neuropathy, and Bickerstaff brainstem encephalitis have been reported under MERS-CoV infection [67]. This virus, however, was never detected in the human CSF. In case of COVID-19 as well, electromyography and other assessments had confirmed occurrence of GBS and axonal neuropathy in infected patients as well [68–70].

3.2 Pathogenic studies and mechanisms in favor of neurotropic nature of the SARS CoV-2

Reports associated with neurological impairments induced by CoV-2 in acute as well as post-acute infection stages have been accumulated since early 2020 [71, 72]. Whether these effects are occurring as a result of the neuroinvasive nature of the virus or due to the overt immune responses is not yet fully understood. A recent study in medRxiv reveals that in comparison to increased inflammatory and cytokine storm markers found in the serum of COVID-19 patients, their levels are rather low in the CSF. This was corroborated by comparing and contrasting the insignificant neuroinflammatory changes in these patients' CSF compared with CSF of patients with autoimmune pathologies that displayed very high neuroinflammation. On the contrary, a significant increase in CSF Neurofilament-L (NF-L) in critical cases suggests neuroaxonal injury and strengthens the neurotropic nature of the virus [73]. The olfactory transmucosal pathway has been an established port of entry for CoV-2 virus, but we only have a limited knowledge about the virus-host interactions [3]. The human sequencing data point toward the role of supporting cells of OE in the viral entry. This is because of the expression of ACE2 and TMPRSS2 proteins in these cells that serve as the entry factors [5]. The immunohistochemical analysis of the SARS-CoV-2 S protein, however, revealed a characteristic granular, perinuclear expression pattern in olfactory mucosal cell types, which were of neuronal origin (revealed by expression of Tuj1, Neurofilament 200, and Olfactory Marker Protein) obtained from the autopsy samples of the COVID-19-infected patients [3]. Additionally, presence of CoV-2 particles was confirmed in the CNS regions including the OB [74]. This questions the current understanding of non-neuronal vs. neuronal viral infection occurring in COVID-19. Generally, neurotropic viruses access the peripheral regions to gain entry into the CNS [75]. Whether the CoV-2 is causing neuronal pathogenesis directly or indirectly is not fully understood. We also do not have a complete understanding of the virus' pathway to the OB and other CNS regions. In an attempt to investigate the pathogenic mechanisms of the virus, an in vitro study of generating human sensory neurons from the human embryonic stem cell lines was carried out. These peripheral sensory neurons were shown to express ACE2 and were indeed receptive to the virus, which is in contrast with the reported non-neuronal expression of ACE2. One hour after the incubation with the virus, intracellular expression of nsp-14, S protein, RdRp, and nucleocapsid phosphoprotein viral genes was substantially upregulated in the infected neurons [76]. The molecular pathologies relating to chemosensory perception were specifically affected in the infected peripheral neurons. The human induced pluripotent stem cells (hIPSCs)-derived midbrain dopaminergic neurons were shown to be selectively permissible to the CoV-2 infection. Further, inflammatory and cellular senescence responses were observed in these neurons both in vitro and upon transplantation in vivo as well [77]. In another attempt to investigate if human neurons are a direct target of this virus, three-dimensional human brain organoids system was utilized. Preferred tropism to mature neurons of the cortical plate in relatively older brain organoids (day 60) was found out. The virus has relatively lesser influence on the actively proliferating neural precursor cells of the ventricular zone of young (day 15) organoids. Moreover, CoV-2-infected neurons displayed mislocalized Tau protein in their soma, which can potentially cause cellular stress reactivity and toxicity [78].

3.3 Evidences of SARS CoV-2 tropism beyond neurons: the other side of the coin

Conflicting results paired with the promiscuous entry routes of the virus stirs the ongoing debate on the neuronal vs. non-neuronal routes of invasion and tropism of SARS-CoV-2 virus. Those who are opposing the axonal hopping of the virus primarily point to the technical limitations of the studies *per se*. Meinhardt and colleagues critically evaluated the imaging data of virus nucleoproteins found at the OE, between the olfactory nerve layer and OB [3]. The axons of OSNs reaching to OB are highly entwined with the ACE2-expressing supporting cells processes, and they reasoned that the immunolabeled imaging may not convincingly reveal whether the virus is present in OSNs or in the wrappings of the sustentacular cells. Nevertheless, ultrastructural assessment using electron microscopy (EM) has also been done both in autoptic human samples and the animal models. Virus-like particles were detected in the cortex of K18-hACE2 mice, and pyknotic cells and abnormal mitochondrial ultra-structures in infected hamsters were observed using Transmission EM (TEM) [79]. A virus cytoplasmic inclusion body was also identified in the OB of an autotoptic patient sample observed using TEM [74]. However, TEM investigations are also subject to aberrant observations as virion-like vesicular bodies can act as decoys for pathologists and that direct neuronal infections cannot be confirmed by using this technique [80]. Post CoV-2 inoculation in rhesus monkey, one group carried out the transcriptomic profiling of the infected cells. Downregulation of genes involved in mitochondrial dysfunctions (ND3, ATP6, and COX3) was observed in the mature neurons, hippocampal microglia, endothelial vascular cells, and oligodendrocytes [81]. This is suggestive of dysfunctions happening in various cell types of the brain, which are collectively leading to CNS abnormalities. Hijacking the lipoprotein metabolism of susceptible cells of the brain barriers (BBB and BSCFB) suggests hematogenous route of entry [82]. Firstly, transcellular route, i.e., entering via ACE2 receptors of the choroid plexus epithelial cells, pericytes, astrocytes lining the endothelial cells followed by weakening of the tight junctions between the vascular endothelial cells of the BBB (called the paracellular modes) and finally utilizing the lipid vesicles and exosomes as the "Trojan horses" to breach the barrier while escaping the host's immune oversight constitutes the non-neuronal mode of entry of the virus [82].

4. Neuro-COVID: neurological consequences of COVID-19 disease

Reports of neurological complications during COVID-19 infection and their persistence after the recovery have accumulated since the early outbreak. These impairments are broadly categorized as Neuro-COVID. Early investigation in China estimated that 36% of the COVID-positive patients had neurological disturbances [83]. Case test studies from France, performed in March–April 2020, also highlighted the occurrence of encephalopathy, state of confusion, and agitation as well as corticospinal tract symptoms in COVID-19 patients admitted to the hospital due to Acute Respiratory Distress Syndrome (ARDS) [84]. Since then, numerous reports and case studies from across different countries have confirmed the prevalence of mild-to-severe neurological and neuropsychiatric in the CoV-2-infected individuals. The neurological impact correlated with the severity of the infection and distributed across the categories of CNS pathologies, peripheral nervous system (PNS) diseases, and/or skeletal muscular disturbances. A cohort-based longitudinal study by the UK Biobank involving multimodal brain imaging before and after the CoV-2 infections

showed emergence of virus-related abnormalities in specific brain regions and cognitive decline upon infection [2]. Using diffusion imaging-based changes as a readout for brain tissue damage upon infection, they observed detrimental effects in regions including the olfactory-limbic areas, the anterior olfactory nucleus, olfactory tubercle, and the anterior piriform cortex. Profound decrease in the gray matter thickness and contrast was also observed in parahippocampal gyrus and lateral orbitofrontal cortex of the patients. Brain abnormalities were more pronounced in hospitalized patients; however, cognitive decline associated with damage to crus II lobule of the cerebellum was found in majority of the individuals who turned positive for the CoV-2 in this longitudinal study [2]. As virus takes the olfactory route, chemosensory impairments are often seen in a large number of infective cases. These impairments, primarily, anosmia and ageusia, have not only served as the robust predictors of the CoV-2 infection, but also affected quality of life of the patients, recovered individuals, and healthcare workers [85, 86]. We will focus on different aspects that influence the severity and durability of the Neuro-COVID symptoms in the following subsections.

4.1 Effect of age and comorbidities on the neurological sequalae

The neurological symptoms tend to vary between older (>60 years of age) and younger (<18 years of age) cohorts. While delirium, myalgia, and fatigue were predominant in older cohorts, the younger cohorts primarily reported smell and taste issues, frequent headaches, and infrequently, seizures [66]. This also hinted toward the possibility of comorbidities playing a role in increasing the severity of the effect of the viral infection and tropism. Patients with preexisting neurological conditions had a higher occurrence of hospitalization, in-hospital mortality, enhanced delirium states, and more overall complications upon suffering from COVID-19 [87, 88]. In fact, social isolation and loneliness associated with the pandemic-induced quarantine added to the mental toll of elderly patients [89]. Symptoms worsened with quarantine in 67.5% patients suffering from Parkinson's disease in a Spanish cohort study [90]. Acute encephalopathy due to infection was more commonly observed in older patients with comorbidities and associated with greater critical care and 30-day mortality chances [87, 91]. Across different levels of the neurological impairments upon CoV-2 infection, age has been shown to be positively correlated with the disease severity [92]. Comorbid conditions including de-myelinating disease, acute encephalopathy, and cerebrovascular disease (CVD) were all positively correlated with the severity of the COVID-19 disease [93]. Patients suffering from Alzheimer's, Parkinson's, and other neurodegenerative disorders are at higher risk from infection and can suffer from greater respiratory, olfactory, and cognitive impairments than others [89, 94]. Elderly individuals also suffer from compromised immunity and increased signs of inflammation (increased cytokines, hormonal changes, decrease in growth factors production) leading to physical and mental frailty [92]. Such multiple dysregulations can lead to age-dependent morbid effects of CoV-2 infection.

4.2 Chemosensory and cognitive impairments: neuro-COVID to Long COVID

Among many neurological impairments, olfactory functioning changes in COVID-19 has highest odds ratio in non-hospitalized cases [86, 95]. These have also become common in healthcare workers. In a study comprising 700 workers, by utilizing a chemosensory perception test, over 80% displayed olfactory and gustatory impairments and ~48% had lowered trigeminal sensitivity. The reduced sensitivity

remained in over 40% of the individuals with olfactory and gustatory impairments and ~23% in those with trigeminal issues [85]. "COVID & Cognition" cross-sectional study continually aims to understand the cognitive deficits in Long COVID [96]. Long COVID comprises long-lasting symptoms and difficulties arising due to COVID-19. Post-acute COVID persists between 3 and 12 weeks while chronic COVID is when the symptoms persist beyond 12 weeks [72]. Cognitive deficits are expected based on the loss of gray matter in specific regions prevalent in COVID-19. A small cohort study of hospitalized patients displayed gray volume reductions in the hippocampus, right amygdala, and left cingulate cortex. Cognitive deficits are thereby likely to occur, and the extent of it depends on the location and mechanism of the neural damage [97]. A multi-domain impact of the infection on human cognition was also observed in a large population, questionnaire-based study using British Intelligence tests. The cognitive deficits in reasoning, problem-solving, spatial planning, and target detection tasks were substantial and persisted post-infection suggesting that cognitive deficits are indeed prevalent in Long COVID [98]. Cognitive blunting, from mild-to-severe, also referred to as "brain fog" has also been observed in Long COVID. Fluorodeoxyglucose-PET study from two COVID-19 patients with confirmed brain fog and cognitive deficits confirmed abnormal hypometabolic regions in anterior cingulate cortex. Hypometabolisms are also observed in other neurological disorders and psychiatric diseases [99]. The prevalence of chemosensory deficits suggests that they can act as predictors of the COVID-19 infection, which has been elaborated upon, in the next section. The advancements in precisely determining neurocognitive deficits along with sensory impairments non-invasively are also explained.

5. Using "olfaction" to detect COVID-19 and the subsequent sensory-cognitive deficits

Having been one of the first symptoms to be affected in COVID-19, our sense of smell became an important and a robust tool to be utilized for detecting if an individual is infected [100]. This became much more beneficial in detecting "asymptomatic" or "paucisymptomatic" patients, i.e., those who did not develop other visible symptoms of the disease [101–103]. Since 2020, many empirical tests that could previously assess olfactory detection and sensitivity and acted as biomarker indicators of neurodegenerative disorders, have been also utilized as early screening tools for COVID-19. Brief Smell Identification test (BSIT), a revised version of University of Pennsylvania Smell Identification test (UPSIT), was among the first self-administered tests, which was utilized during first wave of COVID-19 [104, 105]. Briefly, it consisted of 12 scented strips (encapsulated odors), which are to be scratched by a pencil to release the odor. It is a forced-choice test, and the subject needed to choose one of the four choices which smelled like the tested odor. A high score indicated normal olfactory performance. Using this test, olfactory dysfunction was observed in 40% of the patients. Indeed, in those patients with just olfactory-related problems, other symptoms flared up ~2 days later [105]. Another study used the Persian version of the full 40-odor UPSIT and found out that 98% of the 60 patients had olfactory dysfunctions. In total, 58% of these were either anosmic or severly microsmic [106]. Hummel's quick olfactory sniffing Sticks (q-Sticks) test was also administered in COVID-19 patients, which consisted of asymptomatic individuals as well [107]. This test consisted of recognition of groups of three odors emanating from refill sticks. Although only 14% of the total patients reported smell loss before

the test administration, q-Sticks test revealed that 81% of total patients suffered from anosmia or hyposmia [108]. Since the outbreak, such objective tests that can quantitatively measure olfactory detection, such as Quick-Smell Identification test (Q-SIT), SAFER scent cards, *SCENTinel* 1.0 among others have gained traction [109–111]. However, these traditional tests offer simplistic handle on determining odor detection abilities.

Based on the neurotropic potential of CoV-2, olfactory dysfunctions cannot just be restricted to sensory detection and threshold capacities. Rather, COVID-19 can cause both sensory and cognitive deficits, and efforts are being made to diagnose those too. To this end, highly automated tests with precise stimulus delivery are important.

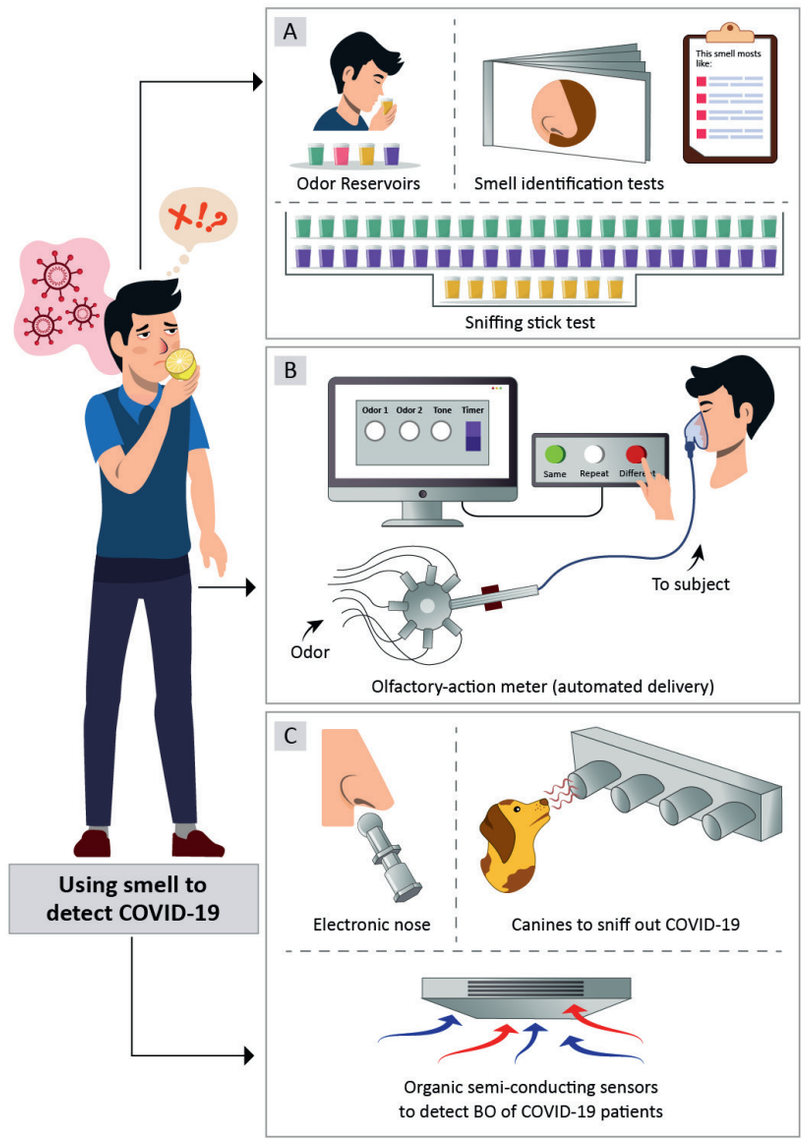

Figure 2.
"Olfaction" as a tool to detect COVID-19. (A) Objective tools such as University of Pennsylvania Smell Identification test (UPSIT), Sniffing stick tests among others have been utilized to evaluate the olfactory detection and discrimination capabilities. These tests consist of delivery of odors to the subject via reservoirs/pen refills/ microencapsulations [104, 106, 115]. (B) An automated odor delivery system, Olfactory-action meter (OAM) has been utilized to precisely calculate odor detection at the threshold levels and olfactory matching skills, i.e., both sensory and cognitive capabilities of symptomatic, asymptomatic, and healthy individuals [101, 112]. (C) Sense of smell, i.e., electronic noses, trained canines, and organic semiconducting sensors are also deployed to using the body odor (BO) of individuals to detect COVID-19 [116–119].

Olfactory-action meter (OAM), an automated machine with custom-written software that can generate odor pulses of varying complexities, has been utilized to assess olfactory detection abilities at differing concentration ranges as well as olfactory matching skills in asymptomatic carriers, symptomatic patients, and those who have recovered from the disease [101, 112, 113]. Compared with normal healthy subjects, up to 81% of the asymptomatic carriers failed at detecting odors at low concentrations (9% (v/v)). In total, 65% of these carriers depicted significantly lower detection at three low-concentration ranges (9–23.1%). Upon administering an olfactory matching task of determining whether the two odors delivered at a set inter-stimulus interval of 5 s are "same" or "different," they found out olfactory working memory deficits in the patients [101]. Not only that, upon carrying out this test with individuals who had recovered from COVID-19 (4–18 months after infection), persistent sensory-cognitive deficits were found out when this paradigm was employed over 5 days [112]. These studies point to the persistence of sensory-cognitive impairments in long-haulers (those suffering from Long-COVID) and also calls for further interrogation of CNS functioning. This also exhibits the importance of monitoring neurocognitive skills during post-infection periods in a pandemic-struck world. These results display the necessity of developing accurate noninvasive methods, which can precisely quantify cognitive deficits in Long COVID [112, 114].

Usage of electronic noses (eNoses) also became popular in detecting COVID-19-infected individuals. eNoses are machines that can mimic animal olfaction and can thus be applied as specific smell detectors of target volatile organic compounds. Usage of an eNose at a drive-through testing station that can detect COVID-19 in real time using body odor that has a nasal passage carried out in an attempt to use them as fast, reliable detectors of this disease [118]. Organic semiconducting sensors could also capture the scent of the asymptomatic carriers of the diseases, suggesting that they can also be deployed at large scale [119]. Finally, dogs can supposedly be our best friends, even during a pandemic. Multiple studies have reported using canines to detect the body odors of the infected patients. Axillary sweat samples of patients may well be successfully discriminated from the normal subjects at a success rate of 76–100% for trained dogs [117]. All these studies thus indicate that sense of smell can be utilized at different levels and scales for diagnosing COVID-19 and furthering the research on cognitive blunting due to this disease (**Figure 2**).

6. Using animal models for mechanistic understanding of entry, invasion, and destruction of nervous system

For a closer interrogation of the role of OE infection by CoV-2, gene expression patterns were studied in the mouse whole olfactory mucosa (WOM) and purified olfactory sensory neurons (OSNs). Single-cell sequencing of mouse WOM uncovered the expression of ACE2 and TMPRSS2 in the dorsally located SUS cells, basal globose cells as well as in a small fraction of the stem cells. Mouse OSNs, however, did not show the expression of the CoV-2 entry genes [6]. Within the OB as well, sequencing did not reveal any neuronal expression of these genes while the immunostaining displayed their expression in the pericytes of OB blood vessels. Postmortem magnetic resonance imaging (MRI) and histopathological examination confirmed microvascular injuries in the OB and brain stem of COVID-19-infected patients [120]. Such studies confirmed the olfactory trans-mucosal pathway of entry of CoV-2 virus into the body [3].

Using the hamster infection model of CoV-2 invasion, nucleoprotein expression was found out in Tuj1-positive infected OSNs and OMP staining also confirmed infection in mature OSNs [121]. Along with local inflammation of OE, SARS-CoV-2 infected the Tuj-1 positive immature OSNs, which appeared to be phagocytosed by the Iba1 and CoV-2-positive immune cells. In fact, global chromatin rearrangements occurred at day 3 post infection, which persisted even after the virus was cleared (10 days post infection) [122]. Whether the interferon response in the OSNs can bring about such chromatin changes in CoV-2 scenario remains an open question. It is also yet to be deciphered if other actively dividing cells are also prone to such disruptions and if not, then what makes certain cell types more unique and susceptible to viral-induced genomic modifications [123].

Nasal irrigation of virus in Golden Syrian Hamsters once again revealed the neuronal invasion. The OMP positive neuronal cilia were vanished in the virus-induced damaged epithelium of the animals. Both neuronal and non-neuronal cells were found out to be positive for cleaved caspase-3 after 4 days of infection, which was suggestive of cell death. Additionally, CoV-2 nucleoprotein was found at the junction of olfactory nerve and the OB and also in previously uncharacterized cells of the glomerular layer of the OB, suggestive of, invasion of the bulb by the virus [124]. Finally, to grasp the molecular underpinnings of the olfactory impairment upon viral infection, transcriptional changes in OE cells of the infected hamsters were studied. A significant reduction in the OR genes, i.e., the genes responsible for olfaction, was observed. At genomic level, they found out a drastic change in the long-range interactions of the OR genes with enhancer/activators 1 day post infection in a non-cell-autonomous fashion.

7. Conclusions

In this chapter, we have discussed the neurotropic nature of the SARS-CoV-2 virus. Using human and animal model studies, research groups across the globe have found out that neuroinvasion can occur by multiple routes, leading to dysfunctions of multiple cell types in the nervous system. The impairments that arise due to infection, which are collectively referred to as the Neuro-COVID, have also been summarized. Some of these symptoms persist in recovered individuals pointing to long-lasting consequences of the infection, which encompass the term Long COVID. We conclude that the virus can play havoc at multiple scales of the nervous system functioning, and the severity depends upon several factors such as the route and extent of infection, variant of the virus, and comorbidities in the patients. Finally, the under-appreciated sense of smell has indeed come into limelight, and the need for quantifying the olfactory and cognitive fitness has become vital during the pandemic. We, hereby, conclude that olfaction can be efficiently used in detecting the infection as well as providing a tool for investigating the cognitive capacities of human beings.

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Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this manuscript.

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References

[1] Puelles VG, Lütgehetmann M, Lindenmeyer MT, Sperhake JP, Wong MN, Allweiss L, et al. Multiorgan and renal tropism of SARS-CoV-2. New England Journal of Medicine. 2020;**383**:590-592

[2] Douaud G, Lee S, Alfaro-Almagro F, Arthofer C, Wang C, McCarthy P, et al. SARS-CoV-2 is associated with changes in brain structure in UK biobank. Nature. 2022;**604**:697-707

[3] Meinhardt J, Radke J, Dittmayer C, Franz J, Thomas C, Mothes R, et al. Olfactory transmucosal SARS-CoV-2 invasion as a port of central nervous system entry in individuals with COVID-19. Nature Neuroscience. 2021;**24**:168-175

[4] Khan M, Yoo S-J, Clijsters M, Backaert W, Vanstapel A, Speleman K, et al. Visualizing in deceased COVID-19 patients how SARS-CoV-2 attacks the respiratory and olfactory mucosae but spares the olfactory bulb. Cell. 2021;**184**:5932-5949

[5] Hoffmann M, Kleine-Weber H, Schroeder S, Krüger N, Herrler T, Erichsen S, et al. SARS-CoV-2 cell entry depends on ACE2 and TMPRSS2 and is blocked by a clinically proven protease inhibitor. Cell. 2020;**181**:271-280

[6] Brann DH, Tsukahara T, Weinreb C, Lipovsek M, den Berge K, Gong B, et al. Non-neuronal expression of SARS-CoV-2 entry genes in the olfactory system suggests mechanisms underlying COVID-19-associated anosmia. Science Advances. 2020;**6**:eabc5801

[7] Fodoulian L, Tuberosa J, Rossier D, Boillat M, Kan C, Pauli V, et al. SARS-CoV-2 receptors and entry genes are expressed in the human olfactory neuroepithelium and brain. iScience. 2020;**23**:101839

[8] Cantuti-Castelvetri L, Ojha R, Pedro LD, Djannatian M, Franz J, Kuivanen S, et al. Neuropilin-1 facilitates SARS-CoV-2 cell entry and infectivity. Science. 1979;**2020**(370):856-860

[9] Kyrou I, Randeva HS, Spandidos DA, Karteris E. Not only ACE2—The quest for additional host cell mediators of SARS-CoV-2 infection: Neuropilin-1 (NRP1) as a novel SARS-CoV-2 host cell entry mediator implicated in COVID-19. Signal Transduction and Targeted Therapy. 2021;**6**:1-3

[10] Huang X, Hussain B, Chang J. Peripheral inflammation and blood–brain barrier disruption: Effects and mechanisms. CNS Neuroscience & Therapeutics. 2021;**27**:36-47

[11] Heald-Sargent T, Gallagher T. Ready, set, fuse! The coronavirus spike protein and acquisition of fusion competence. Viruses. 2012;**4**:557-580

[12] Whittaker GR, Daniel S, Millet JK. Coronavirus entry: How we arrived at SARS-CoV-2. Current Opinion in Virology. 2021;**47**:113-120

[13] Li W, Moore MJ, Vasilieva N, Sui J, Wong SK, Berne MA, et al. Angiotensin-converting enzyme 2 is a functional receptor for the SARS coronavirus. Nature. 2003;**426**:450-454

[14] Gheblawi M, Wang K, Viveiros A, Nguyen Q, Zhong J-C, Turner AJ, et al. Angiotensin-converting enzyme 2: SARS-CoV-2 receptor and regulator of the renin-angiotensin system: Celebrating the 20th anniversary of the

discovery of ACE2. Circulation Research. 2020;**126**:1456-1474

[15] Glowacka I, Bertram S, Müller MA, Allen P, Soilleux E, Pfefferle S, et al. Evidence that TMPRSS2 activates the severe acute respiratory syndrome coronavirus spike protein for membrane fusion and reduces viral control by the humoral immune response. Journal of Virology. 2011;**85**:4122-4134

[16] Jackson CB, Farzan M, Chen B, Choe H. Mechanisms of SARS-CoV-2 entry into cells. Nature Reviews. Molecular Cell Biology. 2022;**23**:3-20

[17] Huang I-C, Bosch BJ, Li F, Li W, Lee KH, Ghiran S, et al. SARS coronavirus, but not human coronavirus NL63, utilizes cathepsin L to infect ACE2-expressing cells. Journal of Biological Chemistry. 2006;**281**:3198-3203

[18] Bayati A, Kumar R, Francis V, McPherson PS. SARS-CoV-2 infects cells after viral entry via clathrin-mediated endocytosis. Journal of Biological Chemistry. 2021;**296**:100306

[19] Whittaker GR. SARS-CoV-2 spike and its adaptable furin cleavage site. Lancet Microbe. 2021;**2**:e488-e489

[20] Sungnak W, Huang N, Bécavin C, Berg M, Queen R, Litvinukova M, et al. SARS-CoV-2 entry factors are highly expressed in nasal epithelial cells together with innate immune genes. Nature Medicine. 2020;**26**:681-687

[21] Klingenstein M, Klingenstein S, Neckel PH, Mack AF, Wagner AP, Kleger A, et al. Evidence of SARS-CoV2 entry protein ACE2 in the human nose and olfactory bulb. Cells, Tissues, Organs. 2020;**209**:155-164

[22] Collin J, Queen R, Zerti D, Dorgau B, Georgiou M, Djidrovski I, et al. Co-expression of SARS-CoV-2 entry genes in the superficial adult human conjunctival, limbal and corneal epithelium suggests an additional route of entry via the ocular surface. The Ocular Surface. 2021;**19**:190-200

[23] Sakaguchi W, Kubota N, Shimizu T, Saruta J, Fuchida S, Kawata A, et al. Existence of SARS-CoV-2 entry molecules in the oral cavity. International Journal of Molecular Sciences. 2020;**21**:6000

[24] Kaya H, Çalışkan A, Okul M, Sarı T, Akbudak İH. Detection of SARS-CoV-2 in the tears and conjunctival secretions of Coronavirus disease 2019 patients. The Journal of Infection in Developing Countries. 2020;(14):977-981

[25] Casagrande M, Fitzek A, Spitzer MS, Püschel K, Glatzel M, Krasemann S, et al. Presence of SARS-CoV-2 RNA in the cornea of viremic patients with COVID-19. JAMA Ophthalmology. 2021;**139**:383-388

[26] Zhou L, Xu Z, Castiglione GM, Soiberman US, Eberhart CG, Duh EJ. ACE2 and TMPRSS2 are expressed on the human ocular surface, suggesting susceptibility to SARS-CoV-2 infection. The Ocular Surface. 2020;**18**:537-544

[27] Huang N, Pérez P, Kato T, Mikami Y, Okuda K, Gilmore RC, et al. SARS-CoV-2 infection of the oral cavity and saliva. Nature Medicine. 2021;**27**:892-903

[28] Brandini DA, Takamiya AS, Thakkar P, Schaller S, Rahat R, Naqvi AR. Covid-19 and oral diseases: Crosstalk, synergy or association? Reviews in Medical Virology. 2021;**31**:e2226

[29] Al-Dalahmah O, Thakur KT, Nordvig AS, Prust ML, Roth W, Lignelli A, et al. Neuronophagia and microglial nodules in a SARS-CoV-2 patient with cerebellar hemorrhage. Acta Neuropathologica Communications. 2020;**8**:1-7

[30] Boldrini M, Canoll PD, Klein RS. How COVID-19 affects the brain. JAMA Psychiatry. 2021;**78**:682-683

[31] Liddelow SA, Guttenplan KA, Clarke LE, Bennett FC, Bohlen CJ, Schirmer L, et al. Neurotoxic reactive astrocytes are induced by activated microglia. Nature. 2017;**541**:481-487

[32] Guo M, Tao W, Flavell RA, Zhu S. Potential intestinal infection and faecal–oral transmission of SARS-CoV-2. Nature Reviews. Gastroenterology & Hepatology. 2021;**18**:269-283

[33] Canetta C, Accordino S, Buscarini E, Benelli G, la Piana G, Scartabellati A, et al. Syncope at SARS-CoV-2 onset. Autonomic Neuroscience. 2020;**229**:102734

[34] Baker J, Incognito A, v, Wilson RJA, Raj SR. Syncope and silent hypoxemia in COVID-19: Implications for the autonomic field. Autonomic Neuroscience. 2021;**235**:102842

[35] Mahammedi A, Ramos A, Bargalló N, Gaskill M, Kapur S, Saba L, et al. Brain and lung imaging correlation in patients with COVID-19: Could the severity of lung disease reflect the prevalence of acute abnormalities on neuroimaging? A global multicenter observational study. American Journal of Neuroradiology. 2021;**42**:1008-1016

[36] Zhou D, Wang Q, Liu H. Coronavirus disease 2019 and the gut–lung axis. International Journal of Infectious Diseases. 2021;113:300-307

[37] Porzionato A, Emmi A, Stocco E, Barbon S, Boscolo-Berto R, Macchi V, et al. The potential role of the carotid body in COVID-19. American Journal of Physiology-Lung Cellular and Molecular Physiology. 2020;**319**:L620-L626

[38] Lionetti V, Bollini S, Coppini R, Gerbino A, Ghigo A, Iaccarino G, et al. Understanding the heart-brain axis response in COVID-19 patients: A suggestive perspective for therapeutic development. Pharmacological Research. 2021;**168**:105581

[39] Nishiga M, Wang DW, Han Y, Lewis DB, Wu JC. COVID-19 and cardiovascular disease: From basic mechanisms to clinical perspectives. Nature Reviews. Cardiology. 2020;**17**:543-558

[40] Bauer L, Laksono BM, de Vrij FMS, Kushner SA, Harschnitz O, van Riel D. The neuroinvasiveness, neurotropism, and neurovirulence of SARS-CoV-2. Trends in Neurosciences. May 2022;**45**(5):358-368

[41] Jakhmola S, Indari O, Chatterjee S, Jha HC. SARS-CoV-2, an underestimated pathogen of the nervous system. SN Comprehensive Clinical Medicine. 2020;**2**:2137-2146

[42] Armstrong L, Collin J, Mostafa I, Queen R, Figueiredo FC, Lako M. In the eye of the storm: SARS-CoV-2 infection and replication at the ocular surface? Stem Cells Translational Medicine. 2021;**10**:976-986

[43] Tremblay M-E, Madore C, Bordeleau M, Tian L, Verkhratsky A. Neuropathobiology of COVID-19: The role for glia. Frontiers in Cellular Neuroscience. 2020;**14**:592214

[44] Barker CF, Billingham RE. Immunologically privileged sites. Advances in Immunology. 1978;**25**:1-54

[45] Han HS, Suk K. The function and integrity of the neurovascular unit rests upon the integration of the vascular and inflammatory cell systems. Current Neurovascular Research. 2005;**2**:409-423

[46] Ludlow M, Kortekaas J, Herden C, Hoffmann B, Tappe D, Trebst C, et al. Neurotropic virus infections as the cause of immediate and delayed neuropathology. Acta Neuropathologica. 2016;**131**:159-184

[47] Kapila CC, Kaul S, Kapur SC, Kalayanam TS, Banerjee D. Neurological and hepatic disorders associated with influenza. British Medical Journal. 1958;**2**:1311

[48] Nichols WG, Peck Campbell AJ, Boeckh M. Respiratory viruses other than influenza virus: Impact and therapeutic advances. Clinical Microbiology Reviews. 2008;**21**:274-290

[49] Bohmwald K, Gálvez NMS, Ríos M, Kalergis AM. Neurologic alterations due to respiratory virus infections. Frontiers in Cellular Neuroscience. 2018;**12**:386

[50] Wallace SJ, Zealley H. Neurological, electroencephalographic, and virological findings in febrile children. Archives of Disease in Childhood. 1970;**45**:611-623

[51] Ann Yeh E, Collins A, Cohen ME, Duffner PK, Faden H. Detection of coronavirus in the central nervous system of a child with acute disseminated encephalomyelitis. Pediatrics. 2004;**113**:e73-e76

[52] Webster DL, Gardner AH, Dye TJ, Chima RS. Status epilepticus: A possible association with human metapneumovirus infection. Pediatrics. 2014;**133**:e747-e750

[53] Desforges M, le Coupanec A, Dubeau P, Bourgouin A, Lajoie L, Dubé M, et al. Human coronaviruses and other respiratory viruses: Underestimated opportunistic pathogens of the central nervous system? Viruses. 2019;**12**:14

[54] Jha NK, Ojha S, Jha SK, Dureja H, Singh SK, Shukla SD, et al. Evidence of coronavirus (CoV) pathogenesis and emerging pathogen SARS-CoV-2 in the nervous system: A review on neurological impairments and manifestations. Journal of Molecular Neuroscience. 2021;**71**:2192-2209

[55] Ye M, Ren Y, Lv T. Encephalitis as a clinical manifestation of COVID-19. Brain, Behavior, and Immunity. 2020;**88**:945

[56] Zhou Z, Kang H, Li S, Zhao X. Understanding the neurotropic characteristics of SARS-CoV-2: From neurological manifestations of COVID-19 to potential neurotropic mechanisms. Journal of Neurology. 2020;**267**:2179-2184

[57] di Mauro G, Scavone C, Rafaniello C, Rossi F, Capuano A. SARS-Cov-2 infection: Response of human immune system and possible implications for the rapid test and treatment. International Immunopharmacology. 2020;**84**:106519

[58] Wu Y, Xu X, Chen Z, Duan J, Hashimoto K, Yang L, et al. Nervous system involvement after infection with COVID-19 and other coronaviruses. Brain, Behavior, and Immunity. 2020;**87**:18-22

[59] Fan H, Tang X, Song Y, Liu P, Chen Y. Influence of COVID-19 on cerebrovascular disease and its possible mechanism. Neuropsychiatric Disease and Treatment. 2020;**16**:1359

[60] Politi LS, Salsano E, Grimaldi M. Magnetic resonance imaging alteration of the brain in a patient with coronavirus disease 2019 (COVID-19) and anosmia. JAMA Neurology. 2020;**77**:1028-1029

[61] von Weyhern CH, Kaufmann I, Neff F, Kremer M. Early evidence of pronounced brain involvement in fatal

COVID-19 outcomes. The Lancet. 2020;**395**:e109

[62] Mehta S, Bhandari S, Mehta S. Brain autopsies in fatal COVID-19 and postulated pathophysiology: More puzzling than a Rubik's cube. Journal of Clinical Pathology. 2021;**74**:612-613

[63] Veleri S. Neurotropism of SARS-CoV-2 and neurological diseases of the central nervous system in COVID-19 patients. Experimental Brain Research. 2022;**240**:9-25

[64] Bergmann CC, Lane TE, Stohlman SA. Coronavirus infection of the central nervous system: host–virus stand-off. Nature Reviews. Microbiology. 2006;**4**:121-132

[65] Murray RS, Brown B, Brain D, Cabirac GF. Detection of coronavirus RNA and antigen in multiple sclerosis brain. Annals of Neurology: Official Journal of the American Neurological Association and the Child Neurology Society. 1992;**31**:525-533

[66] Sharifian-DorcheM,HuotP,OsherovM, Wen D, Saveriano A, Giacomini PS, et al. Neurological complications of coronavirus infection; A comparative review and lessons learned during the COVID-19 pandemic. Journal of the Neurological Sciences. 2020;**417**:117085

[67] Kim J-E, Heo J-H, Kim H, Song S, Park S-S, Park T-H, et al. Neurological complications during treatment of Middle East respiratory syndrome. Journal of Clinical Neurology. 2017;**13**:227-233

[68] Reichard RR, Kashani KB, Boire NA, Constantopoulos E, Guo Y, Lucchinetti CF. Neuropathology of COVID-19: A spectrum of vascular and acute disseminated encephalomyelitis (ADEM)-like pathology. Acta Neuropathologica. 2020;**140**:1-6

[69] Ameres M, Brandstetter S, Toncheva AA, Kabesch M, Leppert D, Kuhle J, et al. Association of neuronal injury blood marker neurofilament light chain with mild-to-moderate COVID-19. Journal of Neurology. 2020;**267**:3476-3478

[70] el Otmani H, el Moutawakil B, Rafai M-A, el Benna N, el Kettani C, Soussi M, et al. Covid-19 and Guillain-Barré syndrome: More than a coincidence! Revue Neurologique (Paris). 2020;**176**:518

[71] Xiong W, Kwan P, Zhou D, del Felice A, Duncan JS, Sander JW. Acute and late neurological complications of COVID19: The quest for evidence. Brain. 2020;**143**:e99-e99

[72] Nalbandian A, Sehgal K, Gupta A, Madhavan MV, McGroder C, Stevens JS, et al. Post-acute COVID-19 syndrome. Nature Medicine. 2021;**27**:601-615

[73] Garcia, Maria A, et al. Cerebrospinal fluid in COVID-19 neurological complications: Neuroaxonal damage, anti-SARS-Cov2 antibodies but no evidence of cytokine storm. Journal of the Neurological Sciences. 2021;**427**:117517

[74] Morbini P, Benazzo M, Verga L, Pagella FGM, Mojoli F, Bruno R, et al. Ultrastructural evidence of direct viral damage to the olfactory complex in patients testing positive for SARS-CoV-2. JAMA Otolaryngology—Head & Neck Surgery. 2020;**146**:972-973

[75] Koyuncu O, Hogue I, Enquist L. Virus infections in the nervous system. Cell Host & Microbe. 2013;**13**:379-393

[76] Lyoo K-S, Kim HM, Lee B, Che YH, Kim S-J, Song D, et al. Direct neuronal

infection of SARS-CoV-2 reveals cellular and molecular pathology of chemosensory impairment of COVID-19 patients. Emerging Microbes & Infections. 2022;**11**:406-411

[77] Han Y, Yang L, Kim TW, Nair MS, Harschnitz O, Wang P, et al. SARS-CoV-2 infection causes dopaminergic neuron senescence. Research Square. 2021. DOI: 10.21203/rs.3.rs-513461/v1

[78] Ramani A, Müller L, Ostermann PN, Gabriel E, Abida-Islam P, Müller-Schiffmann A, et al. SARS-CoV-2 targets neurons of 3D human brain organoids. The EMBO Journal. 2020;**39**:e106230

[79] Zhang L, Zhou L, Bao L, Liu J, Zhu H, Lv Q, et al. SARS-CoV-2 crosses the blood–brain barrier accompanied with basement membrane disruption without tight junctions alteration. Signal Transduction and Targeted Therapy. 2021;**6**:1-12

[80] Hopfer H, Herzig MC, Gosert R, Menter T, Hench J, Tzankov A, et al. Hunting coronavirus by transmission electron microscopy—A guide to SARS-CoV-2-associated ultrastructural pathology in COVID-19 tissues. Histopathology. 2021;**78**:358-370

[81] Jiao L, Yang Y, Yu W, Zhao Y, Long H, Gao J, et al. The olfactory route is a potential way for SARS-CoV-2 to invade the central nervous system of rhesus monkeys. Signal Transduction and Targeted Therapy. 2021;**6**:1-11

[82] Lam SM, Huang X, Shui G. Neurological aspects of SARS-CoV-2 infection—Lipoproteins and exosomes as Trojan horses. Trends in Endocrinology & Metabolism. Aug 2022;**33**(8):554-568

[83] Mao L, Jin H, Wang M, Hu Y, Chen S, He Q, et al. Neurologic manifestations of hospitalized patients with coronavirus disease 2019 in Wuhan, China. JAMA Neurology. 2020;**77**:683-690

[84] Helms J, Kremer S, Merdji H, Clere-Jehl R, Schenck M, Kummerlen C, et al. Neurologic features in severe SARS-CoV-2 infection. New England Journal of Medicine. 2020;**382**:2268-2270

[85] Bussière N, Mei J, Lévesque-Boissonneault C, Blais M, Carazo S, Gros-Louis F, et al. Chemosensory dysfunctions induced by COVID-19 can persist up to 7 months: A study of over 700 healthcare workers. Chemical Senses. 1 Jan 2021;**46**:bjab038. DOI: 10.1093/chemse/bjab038

[86] Gerkin RC, Ohla K, Veldhuizen MG, Joseph PV, Kelly CE, Bakke AJ, et al. Recent smell loss is the best predictor of COVID-19 among individuals with recent respiratory symptoms. Chemical Senses. 1 Jan 2021;**46**:bjaa081. DOI: 10.1093/chemse/bjaa081.

[87] Chou SH-Y, Beghi E, Helbok R, Moro E, Sampson J, Altamirano V, et al. Global incidence of neurological manifestations among patients hospitalized with COVID-19—A report for the GCS-NeuroCOVID consortium and the ENERGY consortium. JAMA Network Open. 2021;**4**:e2112131-e2112131

[88] Jindal HA, Sahoo SS, Jamir L, Kedar A, Sharma S, Bhatt B. Higher coronavirus disease-19 mortality linked to comorbidities: A comparison between low-middle income and high-income countries. Journal of Education Health Promotion. 2021;**10**:377

[89] Palermo S. "Neurocovid": An analysis of the impact of Covid-19 on the older adults. Evolving psychological and neuropsychological understanding. In: Fighting the COVID-19 Pandemic. IntechOpen; 2021. p. 215

[90] Santos-García D, Oreiro M, Pérez P, Fanjul G, Paz González JM, Feal Painceiras MJ, et al. Impact of coronavirus disease 2019 pandemic on Parkinson's disease: A cross-sectional survey of 568 Spanish patients. Movement Disorders. 2020;**35**:1712-1716

[91] Shah VA, Nalleballe K, Zaghlouleh ME, Onteddu S. Acute encephalopathy is associated with worse outcomes in COVID-19 patients. Brain, Behavior, & Immunity—Health. 2020;**8**:100136

[92] Palermo S. Covid-19 pandemic: Maximizing future vaccination treatments considering aging and frailty. Frontiers of Medicine (Lausanne). 2020;**7**:558835

[93] Sullivan BN, Fischer T. Age-associated neurological complications of COVID-19: A systematic review and meta-analysis. Frontiers in Aging Neuroscience. Aug 2021;**13**:374. DOI: 10.3389/fnagi.2021.653694

[94] Brown EE, Kumar S, Rajji TK, Pollock BG, Mulsant BH. Anticipating and mitigating the impact of COVID-19 pandemic on Alzheimer's disease and related dementias. The American Journal of Geriatric Psychiatry. 2020;**28**(7):712-721

[95] Iravani B, Arshamian A, Lundström JN. Loss of olfactory sensitivity is an early and reliable marker for COVID-19. Chemical Senses. 2022;**47**:bjac022. DOI: 10.1093/chemse/bjac022

[96] Guo P, Benito Ballesteros A, Yeung SP, Liu R, Saha A, Curtis L, et al. COVCOG 2: Cognitive and memory deficits in Long COVID: A second publication from the COVID and cognition study. Frontiers in Aging Neuroscience. Mar 2022;**14**. Article ID: 804937. DOI: 10.3389/fnagi.2022.804937

[97] Bougakov D, Podell K, Goldberg E. Multiple neuroinvasive pathways in COVID-19. Molecular Neurobiology. 2021;**58**:564-575

[98] Hampshire A, Trender W, Chamberlain SR, Jolly AE, Grant JE, Patrick F, et al. Cognitive deficits in people who have recovered from COVID-19. EClinicalMedicine. 2021;**39**:101044

[99] Hugon J, Msika E-F, Queneau M, Farid K, Paquet C. Long COVID: Cognitive complaints (brain fog) and dysfunction of the cingulate cortex. Journal of Neurology. 2022;**269**:44-46

[100] Kang YJ, Cho JH, Lee MH, Kim YJ, Park C-S. The diagnostic value of detecting sudden smell loss among asymptomatic COVID-19 patients in early stage: The possible early sign of COVID-19. Auris, Nasus, Larynx. 2020;**47**:565-573

[101] Bhattacharjee AS, Joshi SV, Naik S, Sangle S, Abraham NM. Quantitative assessment of olfactory dysfunction accurately detects asymptomatic COVID-19 carriers. EClinicalMedicine. 2020;**28**:100575

[102] Whitcroft KL, Hummel T. Olfactory dysfunction in COVID-19: Diagnosis and management. Journal of the American Medical Association. 2020;**323**:2512-2514

[103] Li J, Wang X, Zhu C, Lin Z, Xiong N. Affected olfaction in COVID-19: Re-defining "asymptomatic". EClinicalMedicine. Dec 2020;**29-30**:100628

[104] Doty RL, Shaman P, Dann M. Development of the University of Pennsylvania Smell Identification Test: A standardized microencapsulated test of olfactory function. Physiology & Behavior. 1984;**32**:489-502

[105] Ugurlu BN, Akdogan O, Yilmaz YA, Yapar D, Aktar Ugurlu G, Yerlikaya HS, et al. Quantitative evaluation and progress of olfactory dysfunction in COVID-19. European Archives of Oto-Rhino-Laryngology. 2021;**278**:2363-2369

[106] Moein ST, Hashemian SM, Mansourafshar B, Khorram-Tousi A, Tabarsi P, Doty RL. Smell dysfunction: A biomarker for COVID-19. International Forum of Allergy & Rhinology. (Chicago). 2020;**10**(8):944-950

[107] Hummel T, Pfetzing U, Lötsch J. A short olfactory test based on the identification of three odors. Journal of Neurology. 2010;**257**:1316-1321

[108] Bagnasco D, Passalacqua G, Braido F, Tagliabue E, Cosini F, Filauro M, et al. Quick olfactory Sniffin'Sticks test (Q-sticks) for the detection of smell disorders in COVID-19 patients. World Allergy Organization Journal. 2021;**14**:100497

[109] Parma V, Hannum ME, O'Leary M, Pellegrino R, Rawson NE, Reed DR, et al. SCENTinel 1.0: Development of a rapid test to screen for smell loss. Chemical Senses. 1 Jan 2021;**46**:bjab012. DOI: 10.1093/chemse/bjab012

[110] Said M, Davis P, Davis S, Smart K, Davis S, Yan CH. A rapid olfactory test as a potential screening tool for COVID-19. JAMA Otolaryngology—Head & Neck Surgery. 2021;**147**:828-831

[111] Albaharna HA, Alhajjaj AH, al Shabeb HA, Alghanim DA, Alkhabbaz FL, JM AJ. Screening for olfactory dysfunction in COVID-19 patients using quick smell identification test. Indian Journal of Otolaryngology and Head & Neck Surgery. 2021:1-7. DOI: 10.1007/s12070-021-02709-3

[112] Bhowmik R, Pardasani M, Mahajan S, Magar R, Joshi SV, Nair GA, et al. Persistent olfactory learning deficits during and post-COVID-19 infection. 2022. DOI: 10.2139/ssrn.4202478

[113] Abraham NM. Olfactory-action meter for precise quantification of olfactory dysfunctions and neurocognitive deficits. US Patent App. 17/443,527, 2022, Publication number: 20220054075, Feb 24, 2022

[114] Bhowmik R, Pardasani M, Mahajan S, Bhattacharjee AS, Konakamchi S, Phadnis S, et al. Uncertainty revealed by delayed responses during olfactory matching. BioRxiv. 2022. DOI: 10.1101/2022.09.11.507462

[115] Hummel T, Sekinger B, Wolf SR, Pauli E, Kobal G. "Sniffin'sticks": Olfactory performance assessed by the combined testing of odor identification, odor discrimination and olfactory threshold. Chemical Senses. 1997;**22**:39-52

[116] Hag-Ali M, AlShamsi AS, Boeijen L, Mahmmod Y, Manzoor R, Rutten H, et al. The detection dogs test is more sensitive than real-time PCR in screening for SARS-CoV-2. Communications Biology. 2021;**4**:1-7

[117] Grandjean D, Sarkis R, Lecoq-Julien C, Benard A, Roger V, Levesque E, et al. Can the detection dog alert on COVID-19 positive persons by sniffing axillary sweat samples? A proof-of-concept study. PLoS One. 2020;**15**:e0243122

[118] Snitz K, Andelman-Gur M, Pinchover L, Weissgross R, Weissbrod A, Mishor E, et al. Proof of concept for real-time detection of SARS CoV-2 infection with an electronic nose. PLoS One. 2021;**16**:e0252121

[119] Guest C, Dewhirst SY, Lindsay SW, Allen DJ, Aziz S, Baerenbold O, et al.

Using trained dogs and organic semi-conducting sensors to identify asymptomatic and mild SARS-CoV-2 infections: An observational study. Journal of Travel Medicine. 2022;**29**:taac043

[120] Galougahi MK, Ghorbani J, Bakhshayeshkaram M, Naeini AS, Haseli S. Olfactory bulb magnetic resonance imaging in SARS-CoV-2-induced anosmia: The first report. Academic Radiology. 2020;**27**:892

[121] Chan JF-W, Zhang AJ, Yuan S, Poon VK-M, Chan CC-S, Lee AC-Y, et al. Simulation of the clinical and pathological manifestations of coronavirus disease 2019 (COVID-19) in a golden Syrian hamster model: Implications for disease pathogenesis and transmissibility. Clinical Infectious Diseases. 2020;**71**:2428-2446

[122] Zazhytska M, Kodra A, Hoagland DA, Frere J, Fullard JF, Shayya H, et al. Non-cell-autonomous disruption of nuclear architecture as a potential cause of COVID-19-induced anosmia. Cell. 2022;**185**:1052-1064

[123] Sorek M, Meshorer E. Can't smell the virus: SARS-CoV-2, chromatin organization, and anosmia. Developmental Cell. 2022;**57**:1081-1082

[124] de Melo GD, Lazarini F, Levallois S, Hautefort C, Michel V, Larrous F, et al. COVID-19-related anosmia is associated with viral persistence and inflammation in human olfactory epithelium and brain infection in hamsters. Science Translational Medicine. 2021;**13**:eabf8396