# ACS Chemical Neuroscience

Review

# Central Nervous System Targets and Routes for SARS-CoV-2: Current Views and New Hypotheses
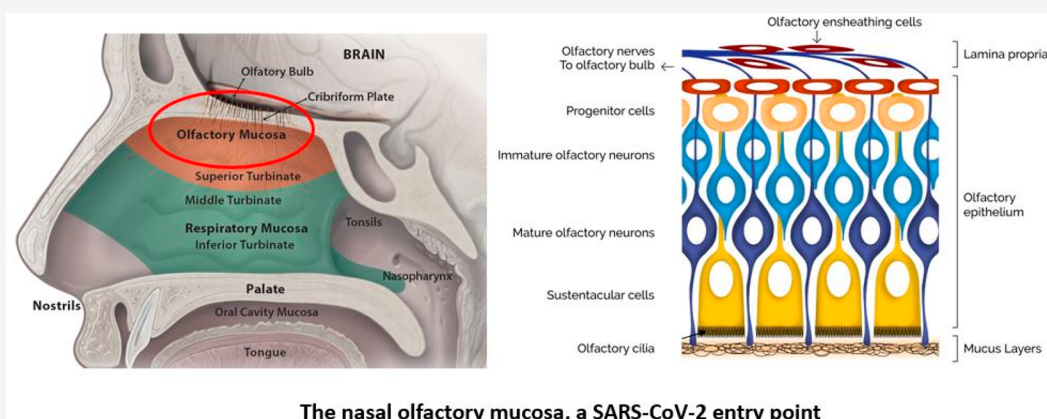
Francisco J. Barrantes*

Cite This: *ACS Chem. Neurosci.* 2020, 11, 2793−2803

Read Online

ACCESS | Metrics & More | Article Recommendations

The nasal olfactory mucosa, a SARS-CoV-2 entry point

**ABSTRACT:** As the coronavirus disease 2019 (COVID-19) pandemic unfolds, neurological signs and symptoms reflect the involvement of targets beyond the primary lung effects. The etiological agent of COVID-19, the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), exhibits neurotropism for central and peripheral nervous systems. Various infective mechanisms and paths can be exploited by the virus to reach the central nervous system, some of which bypass the blood−brain barrier; others alter its integrity. Numerous studies have established beyond doubt that the membrane-bound metalloprotease angiotensin-converting enzyme 2 (ACE2) performs the role of SARS-CoV-2 host-cell receptor. Histochemical studies and more recently transcriptomics of mRNA have dissected the cellular localization of the ACE2 enzyme in various tissues, including the central nervous system. Epithelial cells lining the nasal mucosae, the upper respiratory tract, and the oral cavity, bronchoalveolar cells type II in the pulmonary parenchyma, and intestinal enterocytes display ACE2 binding sites at their cell surfaces, making these epithelial mucosae the most likely viral entry points. Neuronal and glial cells and endothelial cells in the central nervous system also express ACE2. This short review analyzes the known entry points and routes followed by the SARS-CoV-2 to reach the central nervous system and postulates new hypothetical pathways stemming from the enterocytes lining the intestinal lumen.

**KEYWORDS:** COVID-19, SARS-CoV-2, neurotropic virus, angiotensin-converting enzyme 2, ACE2, receptor, brain, viral infection, TMPRSS2

## ■ INTRODUCTION

The *Coronaviridae* are a family of enveloped viruses carrying between 26 and 32 kilobases of single-stranded, positive-sense RNA, the largest so far detected for an RNA virus.[1,2] Coronaviruses (CoVs) infect a wide range of avian and mammalian species. A first set of human CoVs (HCoV-OC43, HCoV-293, HCoV-NL63, and HKU1-CoV) generally causes mild and self-limiting respiratory diseases. A second set of HCoVs are more pathogenic and include the etiological agents of the two epidemics occurring earlier this century, namely, the severe acute respiratory syndrome (SARS-CoV) and the Middle East respiratory syndrome (MERS-CoV) viruses. The ongoing outbreak of coronavirus disease 2019 (COVID-19) is caused by the recently discovered seventh human CoV, the severe acute respiratory syndrome coronavirus 2 virus (SARS-CoV-2).

Various neurotropic viruses exert pathogenic effects on the peripheral and central (CNS) nervous systems. To reach these targets, viruses use different strategies adapted in the course of evolution to exploit cell-surface molecules normally fulfilling
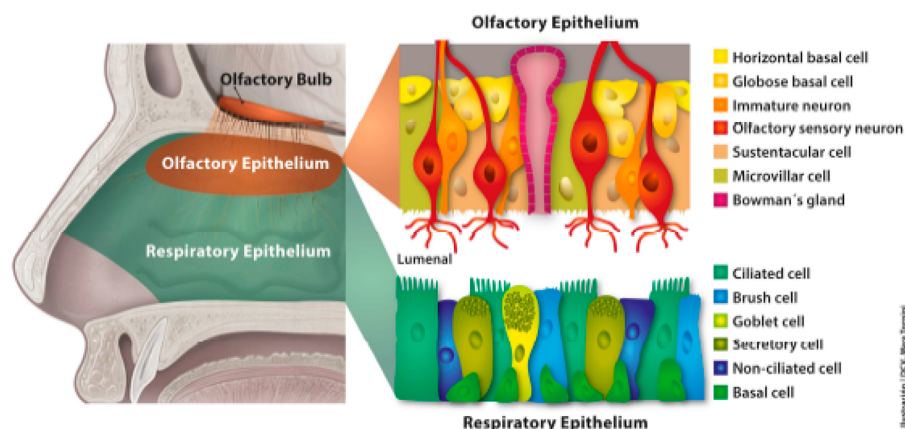
**Figure 1.** Nasal mucosae (left panel) and cellular composition of the olfactory and respiratory regions of the nasal mucosae (right panel). Color code depicts the various cellular components, some of which may be used by SARS-CoV-2 to produce the primary infection and serve as a starting point to reach the brain by neural or non-neural paths.

completely different functions in the cell, to serve as binding partners. Binding of viruses to these adapted molecules, usually transmembrane enzymes, is the first step in viral infection. CoVs have perfected these strategies using metalloproteases as recognition molecules and other membrane-bound enzymes such as the transmembrane serine protease 2 (TMPRSS2) for activation of a key viral protein during the subsequent step of the infection process. SARS-CoV-2 engages its spike S glycoprotein, interacting in sequential order with these two proteins before fusing with the host membrane.[3]

Other viruses such as the mouse hepatitis virus (MHV) beta-CoV rely on the S protein N-term domain to bind to the carcinoembryonic antigen-related cell adhesion molecule 1 (CEACAM1).[4] A zinc metalloprotease, aminopeptidase N (APN, CD13), serves as a host-cell receptor molecule for human H229E-CoV, transmissible gastroenteritis virus, porcine epidemic diarrhea, and feline infectious peritonitis virus. The enzyme dipeptidyl-peptidase 4 (DPP4), also known as a cluster of differentiation 26 (CD26), present in the apical surface of unciliated bronchial epithelial cells, acts as the receptor for MERS-CoV.[5] MERS-CoV can also infect human pulmonary epithelial cells through highly specific but low affinity interactions with sialic acid residues present in host cell-surface glycoproteins[6] using a different region of its spike protein S.[7]

During the first weeks of the pandemic, individuals admitted to hospital presented the typical initial symptomatology of lower respiratory tract infection, associated with fever, fatigue with or without myalgia, sore throat, shortness of breath, dry cough, and in moderate-to-severe cases, dyspnea; rhinorrhea was seldom observed.[8,9] Fever was by far the most frequent sign.[9,10] Computer tomography revealed ground-glass opacity in roughly 50% of hospitalized patients with lower respiratory tract symptoms.[10−12] As the number of cases rose worldwide, the spectrum of symptoms reported for COVID-19 patients widened (see reviews, e.g., in refs 13 and 14). The degree of severity covered a wide range of clinical presentations that were not restricted to the respiratory tract or the pulmonary symptoms and signs. Patients with comorbidities such as hypertension, cardiovascular diseases, or diabetes were found to be more prone to develop severe forms of the disease and multiple organ dysfunction.[12,15,16]

Early reports of sensory dysfunction such as hyposmia[17,18] and other forms of dysosmias and dysgeusias[18−24] were the initial indications of nervous system involvement in COVID-19. These sensory dysfunctions were also observed in patients who recovered from COVID-19 and at later stages presented anosmia, rarely hyposmia.[25] Life-threatening neurological presentations, such as stroke, were and remain exceptional findings.[26]

Reports of neurological complications of COVID-19 became more frequent as the number of hospitalized patients increased. These included cases of peripheral neuropathies and neuromuscular pathologies such as rhabdomyolysis or Guillain-Barré syndrome, to severe CNS complications such as encephalitis, encephalopathy, necrotizing hemorrhagic encephalopathy, some forms of epilepsy, or stroke (reviewed in refs 27−30). The incidence of some of these neurological clinical pictures appears to be relatively high: Mao and co-workers[18] reported that 36.4% of COVID-19 patients showed neurological symptoms, with specific but mild symptoms such as dysgeusias or dysosmias early on in the course of the disease. The reader is referred to several other reviews on the clinical and neurological manifestations of COVID-19 that have recently appeared.[14,24,29,31−39]

The occurrence of neurological manifestations in a viral infectious disease such as COVID-19 poses several interesting issues on the pathogenesis of this clinical entity. Since the first step of the viral infection—binding to a target host cell-surface molecule—mimics a ligand−receptor interaction, the question arises as to which tissues are the ports of entry and in which cells the receptors are located; second, which are the routes followed by the virions surpassing these first barriers to reach the CNS and produce neurological manifestations. This short review addresses these issues: the possible neural or non-neural routes and mechanisms that the SARS-CoV-2 virus could follow to reach and infect the CNS, the presence and distribution of the counterpart cell-host receptor for the SARS-CoV-2 (the membrane-bound metalloprotease angiotensin-converting enzyme 2, ACE2), as analyzed by immunohistochemical or more recently by mRNA transcriptomics, and finally a discussion of hypothetical new routes that the virus could follow to reach the CNS from its enteric entry point.

## ■ THE NASAL MUCOSA

The nasal cavity can be divided into three regions: the squamous region and the respiratory and the sensory olfactory mucosae.

The squamous region, the most external portion, is lined with stratified keratinized squamous epithelium and is therefore unlikely to be a site of viral infection under normal conditions. Most of the nasal cavity corresponds to the respiratory region, which is no longer a squamous multilayered epithelium but a non-keratinized pseudostratified epithelium lined with basal cells, ciliated and nonciliated columnar cells, secretory cells, and goblet cells (Figure 1).[40,41] Basal cells are in contact with the basal membrane and have the inherent capacity to differentiate into the other types in the nasal mucosa. Columnar cells possess cilia and numerous microvilli at their apical surface and are "sealed" with tight junctions at the border between their apical zone and the uppermost section of the basolateral membrane. The goblet cells secrete mucin, a key component of the mucus layer. The respiratory region of the nasal cavity (Figure 1) is innervated by the trigeminal nerve, which stems from the pons in the brainstem[42] and constitutes therefore a potential pathway for the centripetal routing of an infective agent.

The olfactory region is located cephalically to the respiratory region, in and beneath the roof of each nasal cavity, and is lined with a specialized type of pseudostratified monolayer of epithelial cells, harboring (a) olfactory sensory (receptor) neurons, the first cells in the chain of olfactory sensory function, with an exquisite variety of odor receptors expressed at their apical (dendritic) cilia; (b) sustentacular ("supporting") cells, which keep the electrolytic balance required for olfactory function, and as we will see constitute a possible gateway for SARS-CoV-2 entry into the CNS; (c) microvillar cells as in the respiratory region; (d) globose basal cells, which are stem cells that differentiate into olfactory neurons to replace those physiologically lost by aging, and (e) horizontal basal cells, also of stem lineage, that actively generate multiple mature cells in the olfactory epithelium, including the olfactory neuronal and non-neuronal cells.[43] Olfactory stem cells generate support cells in the absence of cell division.[44] The Bowman gland cells also contribute to mucus secretion and homeostatic electrolyte balance. Two major local effects of infection with human CoVs on the nasal mucosa are the disruption of the ciliated epithelium and ciliary dyskinesia, which affect mucociliary clearance and contribute to the pulmonary disease.[45]

Recent single-cell RNA-Seq profiling analysis explored the cellular composition, distribution, and transcriptional heterogeneity of normal human airway mucosa obtained by bronchoscopic aspiration along the respiratory tract.[46] This resulted in an atlas (in the framework of the Human Cell Atlas, https://www.humancellatlas.org/) showing a rather stable cell type-specific gene expression all along the respiratory tract, but exhibiting differential gene expression between otherwise identical cell types from the nasal epithelium down to the 12th division of the tracheobronchial airway epithelium. In addition, rare pulmonary neuroendocrine and brush cells and ionocytes were found to derive from a common population of precursor cells.[46] The same authors explored in more detail the differentiation of the mucociliary system in another single-cell RNA-Seq transcriptomics study on cell cultures of human mucosal cells.[47] They found that goblet cells can be precursors of multiciliated cells and that a subgroup of multiciliated cells expressed the *DEUP1* gene, a hallmark of massive centriole amplification at these cells.

Some of the RNA-Seq studies indicate that the transmembrane proteases purported to be the cell-host receptors for SARS-CoV-2 (see section below) are present in the respiratory epithelial cells,[3,48] whereas other studies show only low level expression of these two proteases in the human mucosal epithelial cells.[49] This latter study identified 194 nonolfactory genes. The human genome contains 857 olfactory receptor genes, of which roughly half are pseudogenes.

## HUMAN VIRAL INFECTIONS OF THE NASAL MUCOSA

Rhinitis and rhinosinusitis (sometimes with concomitant polyposis), the predominant causes of olfactory impairment, were already the most common chronic medical conditions almost two decades ago, affecting in the USA alone about 32 million individuals, with permanent dysfunction predominantly observed in the elderly.[50] Acute viral rhinitis is the most common cause of nontraumatic olfactory dysfunction; traumatic causes are usually associated with head injuries causing tearing or severing of olfactory neuron axons at the cribriform plate. CoVs have been known to be infective agents of the nasal mucosa for many decades, and verification was provided by early experimental clinical studies. An example of this was the reported elevated olfactory thresholds to 1-butanol among volunteers who had been inoculated with one of the human CoVs responsible for the common cold, HCoV-229E, and who developed viral rhinitis relative to uninfected control subjects.[51]

Immunohistochemical studies have documented the alterations in olfactory epithelial cells as sequelae of various types of olfactory disorders following viral infections.[52] The disability of the upper respiratory tract associated with viral diseases gave rise to a nosological entity, the postviral olfactory dysfunction (PVOD); rhinoviruses, belonging to a genus of the *Picornaviridae* family and etiological agent among other viruses of the common cold, are the most frequently isolated species, together with CoVs, parainfluenza virus Epstein—Barr virus, adenoviruses, enteroviruses, and respiratory syncytial viruses.[53,54] Unlike these other viral rhinitides, the olfactory disability observed in several clinical cases of COVID-19 does not usually present rhinorrhea.[20]

## CELLULAR TOPOGRAPHY OF RECEPTOR MOLECULES FOR SARS-COV-2

Both SARS-CoV and SARS-CoV-2 exhibit marked tropism for cells that harbor ACE2 at their plasmalemma, predominantly in cells lining the oral and nasal cavities, upper respiratory tract, and bronchoalveolar cells.[55−57] Other receptors and coreceptor have been postulated for SARS-CoV-2, such as CD147, also known as Basigin or EMMPRIN, a transmembrane glycoprotein belonging to the immunoglobulin superfamily.[58] The transmembrane serine proteases TMRSS2 and TMPRSS4 play a key role in facilitating SARS-CoV-2 spike fusogenic activity on the host-cell plasma membrane, thus promoting the entry of the virus into the cell,[59] acting as "co-receptors" of ACE2.

Why have the nasal and oral mucosae acquired such relevance in the context of COVID-19? At first glance, the contribution of the two mucosae does not appear to lend support to their possible role as viral reservoirs or massive sources of virions for secondary infections. The nasal mucosae cover a surface of $\sim$150−160 cm$^2$ (refs 40 and 60) and the oral cavity $\sim$215 cm$^2$ (ref 61). The tongue expresses much higher amounts of ACE2 than the rest of the oral mucosa.[56] In contrast, the total surface of the intestinal mucosae ($\sim$ 250 m$^2$) or the upper respiratory tract and the pulmonary alveolar region (118 $\pm$ 22 m$^2$ and 91 $\pm$ 18 m$^2$ in male and female individuals, respectively (Colebatch and Ng 1992)) are several orders of magnitude larger. Despite their

relatively small surface, however, the nasal mucosae are of particular importance because of their anatomical vicinity and connections to the forebrain via the shortest of the cranial nerves, the olfactory nerve.

The oral and nasal mucosae became relevant within the context of the current pandemic following the observation of clinical symptoms in COVID-19 associated with alterations in the sensory systems of odor and taste, namely, dysosmias and disgeusias.[21,23,32] Knowledge of the distribution, absolute number, and surface density of ACE2 viral receptor molecules constitutes the first step toward establishing which cells exhibit higher tropism for the viruses, are at greater risk of being infected, and ultimately offer better chances for the viruses to use them as entry points and routes to infecting other organs. The tropism of the virus for certain cells over others also points not only to them being acute targets for gaining entry into the organism but also to their preferential exploitation as reservoirs, as is the case with viruses that reemerge after long latencies. It became important to discriminate whether these sensory disturbances were a manifestation of a peripheral affectation of the mucosae or a more serious neurological complication involving the CNS. It was soon hypothesized that the olfactory epithelium was the likely site of enhanced binding of SARS-CoV-2, correlating this with the clinical olfactory dysfunction observed in some COVID-19 patients, and the possibility was suggested that the olfactory receptor neurons were the site of origin of subsequent brain infection by the virus.[62] Although the early clinical data did not reveal a high incidence of severe neurological complications in COVID-19, such as encephalopathies or encephalitis,[27,29] as the pandemic progressed over time so did the casuistic involving the CNS.[35]

In parallel, experimental data began to emerge. Using a mouse animal model, the predominant expression of ACE2 and TMPRSS2 in the sustentacular cells of the olfactory epithelium was demonstrated, thus suggesting that non-neuronal cells could be responsible for the olfactory impairment.[63] This was followed by experimental demonstration that this was indeed the case: massive damage of the olfactory epithelium was observed as early as 2 days after nasal instillation of SARS-CoV-2 in golden Syrian hamsters, with a substantial loss of cilia.[64] The injured cells were primarily the sustentacular cells.

As is the case with other cell-surface receptors, the distribution and local density of ACE2 play a determinant role in the efficacy of the binding step, be it ligand or virion, especially if ACE2 forms supramolecular aggregates that enhance the chances of successful hits by the viral particle. Clustered ACE2 molecules in complex with MERS-CoV have been experimentally observed.[65]

Transcriptomic RNA-Seq analyses have shown that respiratory epithelial cells express ACE2 and TMPRSS2, the transmembrane serine protease SS 2 required for viral S protein activation, albeit in variable amounts.[3,48,49,59] The situation differs in the olfactory region of the nasal mucosa, where only TMPRSS2 appears to be present in both immature olfactory neurons and non-neuronal cells in mice.[66,67] In the latter study, essentially all (98.9%) olfactory receptor genes were found to be expressed in mature olfactory sensory neurons.

An RNA-Seq study found that goblet, basal, and ciliated cells in the respiratory region of the nasal mucosa (Figure 1) express high levels of the ACE2 and TMPRSS2 genes, together with genes involved in innate immune functions and antiviral genes (*IDO1, IRAK3, NOS2, TNFSF10, OAS1,* and *MX1*), suggesting that these cell types could serve as entry points for SARS-CoV-2 infection and, not less importantly, viral reservoirs for

dissemination. Highest expression was found in goblet (especially goblet 2 cells) and ciliated cells.[68] The *TMPRSS2* gene was only expressed in a subset of *ACE2*+ cells, suggesting that the virus might use alternative pathways.[68] TMPRSS2- cells could instead use cathepsin B/L as a substitute membrane-bound enzyme co-opted as receptor.[3] Also applying bulk and single-cell RNA-Seq methods, another study analyzed the cell types present in the olfactory epithelium and olfactory bulb that expressed the *ACE2* and *TMPRSS2* genes.[69] Remarkably, neither olfactory sensory neurons nor olfactory bulb neurons express the SARS-CoV-2 host-cell receptors. Instead, the gene coding for the ACE2 protein in the olfactory bulb were only found in the vascular pericytes, the cells involved in maintaining blood pressure regulation and the integrity of the BBB, as well as in olfactory support cells and stem cells. *TMPRSS2* was not expressed in the olfactory bulb. The authors surmise that it is the infection of the non-neuronal cells that contributes to the olfactory dysfunction of COVID-19 patients. This opens up the possibility that once the epithelial cell barrier is surpassed, the virions enter local capillaries in the nasal submucosa and make their way into the capillary lumen via the pericytes, rich in ACE2, and the endothelial cell, also rich in ACE2,[70] to reach general circulation.

An in vitro study using organoids of human airway epithelium found that SARS-CoV-2 readily infected *ciliated cells* but not goblet cells.[71] These findings contrast with those of another study in which *ACE2+TMPRSS2+* gene coexpressing cells were found in nasal *goblet* secretory cells, *AT2 alveolar* cells, and ileal absorptive *enterocytes*. By treating primary human upper airway basal cells with distinct types of inflammatory cytokines, or infecting cells with human influenza virus, the authors further showed that the *ACE2+* gene is stimulated by human interferon-$\alpha$.[72] In one of the most comprehensive analyses to date, RNA-Seq libraries compiled from ~29 000 single cells from human olfactory neuroepithelium found expression of *ACE2* and *TMPRSS2* genes in *sustentacular* cells, and expression of the ACE2 protein alone was observed in a subset of these cells.[73] Olfactory sensory neurons showed little or no expression of the two proteins.

A note of caution comes from a study drawing a comparison between the transcriptomic mRNA profile and the immunocytochemical protein expression profile of ACE2 in more than 150 cell types. The highest expression of ACE2 was observed in the intestinal tract, with brain showing no expression, and low or no expression in a subset of cells in the respiratory system.[57] The study also reanalyzed the transcriptomics profiles of 9 other studies in the literature, confirming both the high expression levels (>60%) in ileal *enterocytes* and the enrichment of ACE2 in AT2 cells in the pulmonary parenchyma.

Heterogeneity in the expression of ACE2 missense mutants in different ethnic groups was recently reported,[74] providing another source of variability in risk among COVID-19 patients and the different susceptibility of certain organs to become targets of the disease. RNA-Seq data extracted from more than 4 million human cells by the Human Cell Atlas project identified subsets of respiratory epithelial cells in the nasal passages, airways, and alveoli coexpressing both ACE2 and the protease (*ACE2+TMPRSS2+*).[75] Coexpression in enterocytes, corneal epithelial cells, cardiomyocytes, heart pericytes, olfactory sustentacular cells, and renal epithelial cells may provide higher-susceptibility targets for the virus. Furthermore, some of these *ACE2+TMPRSS2+* gene-expressing cells were found to share a gene expression program that mediates viral entry and
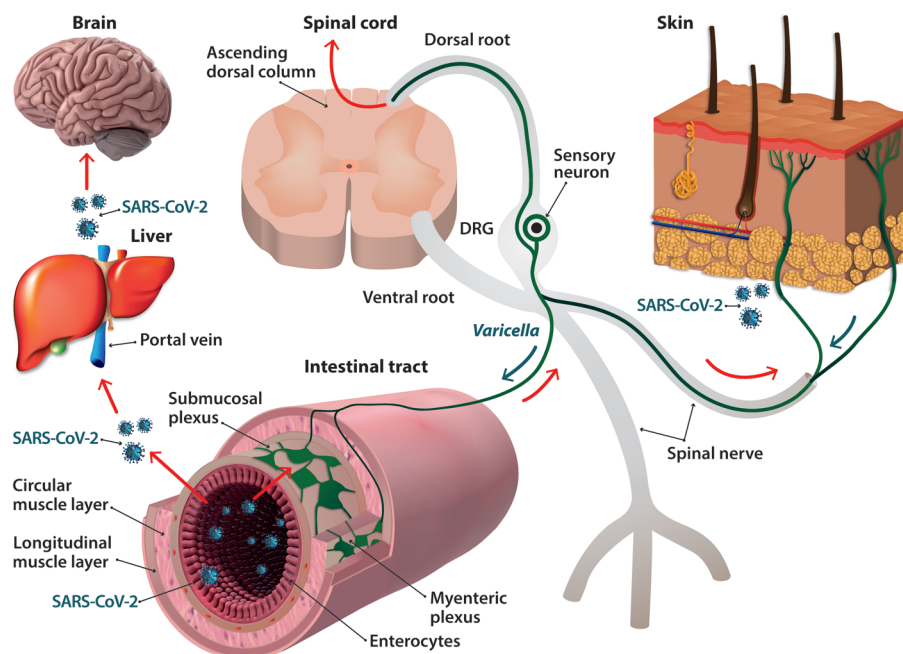
**Figure 2.** Hypothetical alternative SARS-CoV-2 routes in addition to the ones originating in the nasal mucosa (Figure 1). These routes stem from a common *entry point*: the enterocyte lining the intestinal lumen (bottom left corner of the diagram). Upon enterocyte or para-enterocyte (tight junction) infection, the virus may gain access to (a) the submucosal capillary network of the portal vein system (red arrows at the left bottom of the scheme) to reach the liver and subsequently the brain via a *hematogenous route*. (b) A second hematogenous route, following infection of inflammatory cells in the submucosal connective tissue to eventually reach the CNS via a "Trojan horse" mechanism has been observed with some CoVs in other human respiratory diseases.[101] (c) I also suggest the possibility that SARS-CoV-2 may utilize a third neural route resulting from the infection of submucosal plexus and/or myenteric plexus neurons to deliver virions into the CNS via a neuron-to-neuron track[102] following the dorsal root ganglion sensory neuron retrograde path (see main text).

immune functions, like genes coding for IL-6, its receptor IL-6R, and coreceptor IL-1R.[75] In children, T2 inflammation and interferon response to respiratory viruses upregulate the *ACE2* and *TMPRSS2* genes through other interleukins such as IL-13.[76] However, interferon differentially increases only ACE2 in asthmatic patients,[77] highlighting the variability of interleukin regulation of gene expression.

A recent immunocytochemical study found expression of ACE2 in the motile cilia of the respiratory tract epithelia and demonstrated that among various factors, comorbidities modify the expression of the enzyme.[78] One such comorbidity is smoking, a habit that alters the morphology of cilia and mucociliary clearance.[79] Using reverse genetics, a green fluorescent protein (GFP)-reporter virus was used as a probe to investigate SARS-CoV-2 pathogenesis along the respiratory tract. The highest expression was found in ciliated epithelial cells of the nasal mucosa with decreasing expression along the lower tract, paralleled by a similar pattern in viral infectivity in cell cultures; in pulmonary tissue, ciliated cells and AT2 pneumocytes showed expression, though much lower than in nasal mucosa.[80] Mice placed for 5 months under regimes of exposure to cigarette smoke showed a dose-dependent increase in pulmonary ACE2 levels, up to ~80% higher in animals subjected to the maximal dose; a similar dose-dependent expression was observed in humans, with an associated expansion of the number of ACE2-rich mucous-secreting goblet cells.[81]

One remarkable aspect of the COVID-19 pandemic is that right from its outbreak it triggered a wide variety of studies conducted at an accelerated pace and focused on different aspects of the disease, from the clinical to the more basic extremes of the research spectrum. The transcriptomics approaches discussed in this section reflect the contribution of the latter, with the converging expertise of high-throughput next generation sequencing (NGS) RNA-Seq techniques, specialized *ad hoc* software technology, in silico analyses of large library data banks, and single-cell cytology approaches to map the static cellular transcriptome. The implementation of these approaches has led to the rapid identification of the phenotypic distribution of the host-cell receptor of SARS-CoV-2, the metalloenzyme ACE2, in epithelial mucosae, and the coreceptor protease TMPRSS2. A most interesting outcome is the heterogeneous distribution revealed by the single-cell analyses and not apparent (or averaged) in population-level studies, depicting subsets of epithelial cellular phenotypes. These transcriptional signatures may prove important in understanding the susceptibility of certain cells to infection, virion-mammalian cell interactions, the characteristics of the virion replication cycle in different cell types, and in devising prophylactic or therapeutic strategies for this and other viral diseases.

## ACE2 IN THE CNS

ACE2 is a key element in the anti-inflammatory and hypotensive arm of the renin-angiotensin-aldosterone system (RAAS). An endogenous RAAS is operative in the CNS.[82−84] This system, which has an important role in the brain, has two branches: the vasoconstrictor and pro-inflammatory renin-angiotensin [1-9]-angiotensin converting enzyme (ACE) branch and the vaso-dilator and anti-inflammatory ACE2-angiotensin [1-7]-Mas receptor arm. ACE2 enzymatically modifies the vasoconstrictor peptides angiotensin II and angiotensin I into the vasodilator peptides, Ang [1-7] and Ang [1-9], respectively. Angiotensin [1-7] (Ang[1-7]) is the predominant form in various regions of the brain, including the hypothalamus and amygdala, as well as in

the medulla oblongata.[85] Ang[1-7] inhibits hypothalamic noradrenergic neurotransmission, reducing inflammation, oxidative stress, and neuronal apoptosis (see literature meta-analysis in ref 86).

Potential sites for SARS-CoV-2 recognition in brain can be inferred from the *ACE2* mRNA distribution in adult human brain. ACE2 has been found to be highly expressed in the substantia nigra, choroid plexus, ventricles, middle temporal gyrus, posterior cingulate cortex, and olfactory bulb.[70] The protein is expressed in the cytoplasm of both neuronal and glial cells of human brain, in sympathetic tracts of the brainstem, and in the motor cortex.[82] Transcriptomic analyses have found ACE2 to be highly expressed in both excitatory and inhibitory neurons, astrocytes, oligodendrocytes, and endothelial cells.[70] Interestingly, ACE2 has also been found in the brainstem cardiorespiratory nuclei,[83,87] raising the possibility that the direct SARS-CoV-2 attack on these centers is responsible for the atypical form of the acute respiratory distress like-syndrome (ARDS) that characterizes some terminal forms of the disease, now redefined as ARDS-like syndrome in COVID-19 or "CARDS".[88,89]

The widespread distribution of the host-cell receptor for SARS-CoV-2 in the CNS can be correlated with the symptomatology of some of the neurological presentations and/or complications of COVID-19. Data from autopsies are still scarce. A most recent study of 32 COVID-19 autopsies showed SARS-CoV-2 RNA in the respiratory and cardiovascular regulatory centers in the medulla oblongata.[90] Further data are required to fully substantiate the direct attack on the CNS or its involvement as a cause of death, although some cases of severe encephalitis and encephalopathies as neurological complications have been reported.[91−93]

## ALTERNATIVE HYPOTHETICAL ROUTES THAT SARS-COV-2 MAY FOLLOW TO REACH THE CNS

Using the nasal mucosa as its *entry point* and primary infection site, SARS-CoV-2 can gain access to the brain parenchyma following the various short-path neural or hematogenous routes that link these mucosae to the anatomically adjacent forebrain across the cribriform plate, as analyzed previously (Figure 1), but additional viral *entry points* need be considered because of their much larger areas, abundance of SARS-CoV-2 receptors and coreceptors, and hence important virion replicative capacity. These are the respiratory system (tract epithelium-to-capillary or alveolar pneumocyte cell-to capillary) leading to virus passage to the pulmonary/general circulation (which will not be dealt with) and the gastrointestinal tract (Figure 2). In addition, I discuss here *hypothetical routes* relating to the latter.

The intestinal (predominantly the small intestine) lumen *entry point* is a monolayer of cylindrical epithelium with a predominant cell phenotype, the enterocyte, covering a surface close to 250 m$^2$ in contrast to the ~150 sq. cm of the nasal mucosae. Virus infection proceeds at the apical surface of the enterocyte, covered with microvilli that increase the absorptive area (relative to a flat surface) ~25 times and which concomitantly amplifies the coverage with ACE2 receptor molecules. The gene coding for this receptor, *ACE2*, is expressed together with its coreceptor, TMPRSS2 in absorptive enterocytes in human ileum[72] together with TMPRSS4.[59]

Although SARS-CoV-2 internalization is still not fully understood, other CoVs employ both endocytic and non-endocytic mechanisms.[94] MHV-2, for instance, is internalized by a clathrin-mediated, Eps15-independent mechanism.[95] SARS-

CoV-2 pseudovirions were recently shown to be internalized in mammalian cells in vitro mainly by an endocytic mechanism dependent on the protease cathepsin L, the lysosomal downstream effector pore channel subtype 2 (TPC2), and phosphatidylinositol-3,5-bisphosphate (PI(3,5)P2). The inositol lipid is synthesized by phosphatidylinositol 3-phosphate 5-kinase (PIKfyve) in the early endosome, making this enzyme a potential drug target for SARS-CoV-2 inhibition.[48] An example of a nonendocytic internalization mechanism is the passage of virions between epithelial intercellular junctions.[96]

Once inside the enterocyte, the virus undergoes its replicative cycle, and virion shedding across the enterocyte basal membrane puts SARS-CoV-2 in contact with the rich and extensive capillary network in the intestinal villi, only tens of microns from the epithelial lining. Pericytes[69] and endothelial cells, rich in ACE2,[70] offer new targets for the virion to reach the general circulation via the hepatic portal system. This first *hematogenous route* (a) is schematically portrayed in Figure 2. Evidence of gastrointestinal disease is observed in 45% of COVID-19 necropsies.[97] Adverse pre-existing endothelial conditions as observed in several comorbidities in COVID-19 patients and/or the effects of hyperimmune response syndrome on the endothelial cell bed (cytokine release syndrome)[98] may lay the ground for capillary dysregulation supporting SARS-CoV-2 infection of the CNS after defeating a weakened BBB (Figure 2).

(b) Having reached the general circulation, SARS-CoV-2 may also employ a second hematogenous route—the so-called "Trojan horse" mechanism—known to be operative for several microbial agents that infect the brain parenchyma,[99] involving extravasation of inflammatory phagocytic cells (leukocytes, mostly monocytes and lymphocytes) into the meninges and cerebrospinal fluid. This route would follow essentially the same course as (a) above, except that it is a facilitated path, because the capillary network in the outer meningeal space is devoid of tight junctions. Furthermore, the meningeal lymphatic system serving the CNS provides additional pathways from the meningeal space to the brain parenchyma.[100]

(c) A third hypothetical *neural route* is that SARS-CoV-2 virions, after surpassing the enteric epithelial wall, could directly infect neuronal cells of the submucosal or myenteric plexus and through neuron-to-neuron transport[102] and also the sympathetic neuron of the dorsal root ganglion, as shown in Figure 2. The viruses would then be able to centripetally reach the CNS via the spinal cord, subsequently propagating either from neuron to neuron synaptically or crossing the blood−cerebrospinal fluid barrier and the choroid plexus, again bypassing the BBB using the Trojan horse mechanism described above. Other neurotropic viruses such as varicella zoster virus[103] employ the DRG sympathetic neuron bidirectionally to reach the CNS and the peripheral sensory nerve endings. Bulk RNA transcriptomic analyses have shown expression of the *ACE2* gene in human DRG neuronal cells[104,105]. Human DRG neurons express *MRGPRD* and *Nppb* genes, the former of which is selectively expressed in a subset of nociceptive receptors that forms peripheral nerve endings in colon[106] or meninges[107] together with the *ACE2* gene.[105] The painful peripheral neuropathies observed in some COVID-19 patients could be associated with interferon-1-induced hyperexcitability of DRG neurons resulting from viral infection.[108] A similar exacerbated immune response may also account for the peripheral vascular inflammatory reactions observed in the multisystem Kawasaki-like syndrome that affects some COVID-19 patients,[109,110] particularly children. Dermatological manifestations of COVID-

19 such as maculopapular exanthem present in ∼36% of patients[111] could also progress from the DRG neuron to the skin in an anterograde fashion, as schematically shown in the bottom right portion of Figure 2 (red arrow). For all these reasons, the intestinal mucosa is proposed to be a preferred entry point, major viral reservoir, and favored starting point for neurotropic routes.

## CONCLUDING REMARKS

The distribution and abundance of the ACE2 molecule in different cells dictate the tropism of the virus and probably the viral load in each target surface. In the first part of the review, I analyzed the "cellular cartography" of the receptor molecule in the various cell phenotypes of the nasal and intestinal mucosae based on RNA-Seq analysis. Although there is as yet no universal consensus, the topography and abundance of ACE2 and SARS-CoV-2 coreceptor protein, TMPRSS2, and experiments in animal models raise the possibility that the most likely site of peripheral lesion associated with the dysosmias in COVID-19 is the sustentacular cell, a non-neuronal epithelial cell. Effects on the CNS via the nasal mucosa cannot be discarded, and the ACE2 transcriptomics show a remarkably wide distribution of the receptor in many regions of the brain.

The second part of the review dissects hypothetical routes to the CNS and other targets stemming from a much larger receptive region, the apical plasmalemma of the intestinal epithelial cell, the enterocyte. The intestinal mucosa is proposed to be a preferred entry point, major viral reservoir, and favored starting point for neurotropic routes that could be used by SARS-CoV-2 upon binding to ACE2 and fusing to the enterocyte's apical membrane with the aid of TMPRSS2 and TMRSS4. Examples of the correlation between these postulated routes and some clinical manifestations of COVID-19 are provided.

## AUTHOR INFORMATION

### Corresponding Author
Francisco J. Barrantes — *Institute of Biomedical Research (BIOMED), UCA-CONICET, C1107AFF Buenos Aires, Argentina;* ● orcid.org/0000-0002-4745-681X; Email: francisco_barrantes@uca.edu.ar

Complete contact information is available at:
https://pubs.acs.org/10.1021/acschemneuro.0c00434

### Author Contributions
I conceived and designed the study, searched the literature, interpreted the data, and wrote the manuscript. I conceived the illustrations and had help to produce them.

### Notes
The author declares no competing financial interest.

## ACKNOWLEDGMENTS

## REFERENCES

(1) Su, S., Wong, G., Shi, W., Liu, J., Lai, A. C. K., Zhou, J., Liu, W., Bi, Y., and Gao, G. F. (2016) Epidemiology, Genetic Recombination, and Pathogenesis of Coronaviruses. *Trends Microbiol.* 24 (6), 490−502.

(2) Li, F. (2013) Receptor recognition and cross-species infections of SARS coronavirus. *Antiviral Res.* 100 (1), 246−254.

(3) Hoffmann, M., Kleine-Weber, H., Schroeder, S., Krüger, N., Herrler, T., Erichsen, S., Schiergens, T. S., Herrler, G., Wu, N. H., Nitsche, A., Müller, M. A., Drosten, C., and Pöhlmann, S. (2020) SARS-CoV-2 Cell Entry Depends on ACE2 and TMPRSS2 and Is Blocked by a Clinically Proven Protease Inhibitor. *Cell* 181 (2), 271−280.

(4) Kubo, H., Yamada, Y. K., and Taguchi, F. (1994) Localization of neutralizing epitopes and the receptor-binding site within the amino-terminal 330 amino acids of the murine coronavirus spike protein. *J. Virol.* 68 (9), 5403−5410.

(5) Letko, M., Miazgowicz, K., McMinn, R., Seifert, S. N., Sola, I., Enjuanes, L., Carmody, A., van Doremalen, N., and Munster, V. (2018) Adaptive Evolution of MERS-CoV to Species Variation in DPP4. *Cell Rep.* 24 (7), 1730−1737.

(6) Li, W., Hulswit, R. J. G., Widjaja, I., Raj, V. S., McBride, R., Peng, W., Widagdo, W., Tortorici, M. A., van Dieren, B., Lang, Y., van Lent, J. W. M., Paulson, J. C., de Haan, C. A. M., de Groot, R. J., van Kuppeveld, F. J. M., Haagmans, B. L., and Bosch, B.-J. (2017) Identification of sialic acid-binding function for the Middle East respiratory syndrome coronavirus spike glycoprotein. *Proc. Natl. Acad. Sci. U. S. A.* 114 (40), E8508−E8517.

(7) Park, Y. J., Walls, A. C., Wang, Z., Sauer, M. M., Li, W., Tortorici, M. A., Bosch, B. J., DiMaio, F., and Veesler, D. (2019) Structures of MERS-CoV spike glycoprotein in complex with sialoside attachment receptors. *Nat. Struct. Mol. Biol.* 26 (12), 1151−1157.

(8) Wang, D., Hu, B., Hu, C., Zhu, F., Liu, X., Zhang, J., Wang, B., Xiang, H., Cheng, Z., Xiong, Y., Zhao, Y., Li, Y., Wang, X., and Peng, Z. (2020) Clinical Characteristics of 138 Hospitalized Patients With 2019 Novel Coronavirus−Infected Pneumonia in Wuhan, China. *JAMA* 323 (11), 1061−1069.

(9) Huang, C., Wang, Y., Li, X., Ren, L., Zhao, J., Hu, Y., Zhang, L., Fan, G., Xu, J., Gu, X., Cheng, Z., Yu, T., Xia, J., Wei, Y., Wu, W., Xie, X., Yin, W., Li, H., Liu, M., Xiao, Y., Gao, H., Guo, L., Xie, J., Wang, G., Jiang, R., Gao, Z., Jin, Q., Wang, J., and Cao, B. (2020) Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *Lancet* 395 (10223), 497−506.

(10) Guan, W. J., Ni, Z. Y., Hu, Y., Liang, W. H., Ou, C. Q., He, J. X., Liu, L., Shan, H., Lei, C. L., Hui, D. S. C., Du, B., Li, L. J., Zeng, G., Yuen, K. Y., Chen, R. C., Tang, C. L., Wang, T., Chen, P. Y., Xiang, J., Li, S. Y., Wang, J. L., Liang, Z. J., Peng, Y. X., Wei, L., Liu, Y., Hu, Y. H., Peng, P., Wang, J. M., Liu, J. Y., Chen, Z., Li, G., Zheng, Z. J., Qiu, S. Q., Luo, J., Ye, C. J., Zhu, S. Y., and Zhong, N. S. (2020) Clinical Characteristics of Coronavirus Disease 2019 in China. *N. Engl. J. Med.* 382 (18), 1708−1720.

(11) Argenziano, M. G., Bruce, S. L., Slater, C. L., Tiao, J. R., Baldwin, M. R., Barr, R. G., Chang, B. P., Chau, K. H., Choi, J. J., Gavin, N., Goyal, P., Mills, A. M., Patel, A. A., Romney, M. S., Safford, M. M., Schluger, N. W., Sengupta, S., Sobieszczyk, M. E., Zucker, J. E., Asadourian, P. A., Bell, F. M., Boyd, R., Cohen, M. F., Colquhoun, M. I., Colville, L. A., de Jonge, J. H., Dershowitz, L. B., Dey, S. A., Eiseman, K. A., Girvin, Z. P., Goni, D. T., Harb, A. A., Herzik, N., Householder, S., Karaaslan, L. E., Lee, H., Lieberman, E., Ling, A., Lu, R., Shou, A. Y., Sisti, A. C., Snow, Z. E., Sperring, C. P., Xiong, Y., Zhou, H. W., Natarajan, K., Hripcsak, G., and Chen, R. (2020) Characterization and clinical course of 1000 patients with coronavirus disease 2019 in New York: retrospective case series. *BMJ. (Clinical Res.)* 369, m1996.

(12) Jain, V., and Yuan, J.-M. (2020) Systematic review and meta-analysis of predictive symptoms and comorbidities for severe COVID-19 infection. *Int. J. Public Health* 25, 1−14.

(13) Harapan, H., Itoh, N., Yufika, A., Winardi, W., Keam, S., Te, H., Megawati, D., Hayati, Z., Wagner, A. L., and Mudatsir, M. (2020) Coronavirus disease 2019 (COVID-19): A literature review. *J. Infect. Publ. Health* 13 (5), 667−673.

(14) Rodriguez-Morales, A. J., Cardona-Ospina, J. A., Gutiérrez-Ocampo, E., Villamizar-Peña, R., Holguin-Rivera, Y., Escalera-Antezana, J. P., Alvarado-Arnez, L. E., Bonilla-Aldana, D. K., Franco-Paredes, C., Henao-Martinez, A. F., Paniz-Mondolfi, A., Lagos-Grisales, G. J., Ramírez-Vallejo, E., Suárez, J. A., Zambrano, L. I., Villamil-Gómez,

W. E., Balbin-Ramon, G. J., Rabaan, A. A., Harapan, H., Dhama, K., Nishiura, H., Kataoka, H., Ahmad, T., and Sah, R. (2020) Clinical, laboratory and imaging features of COVID-19: A systematic review and meta-analysis. *Travel medicine and infectious disease 34*, 101623.

(15) Grasselli, G., Zangrillo, A., Zanella, A., Antonelli, M., Cabrini, L., Castelli, A., Cereda, D., Coluccello, A., Foti, G., Fumagalli, R., Iotti, G., Latronico, N., Lorini, L., Merler, S., Natalini, G., Piatti, A., Ranieri, M. V., Scandroglio, A. M., Storti, E., Cecconi, M., Pesenti, A., and Network, f. t. C.-L. I. (2020) Baseline Characteristics and Outcomes of 1591 Patients Infected With SARS-CoV-2 Admitted to ICUs of the Lombardy Region, Italy. *JAMA 323* (16), 1574−1581.

(16) Atkins, J. L., Masoli, J. A., Delgado, J., Pilling, L. C., Kuo, C.-L. C., Kuchel, G., and Melzer, D. Preexisting comorbidities predicting severe COVID-19 in older adults in the UK Biobank Community Cohort. *J. Gerontol., Ser. A* 2020, DOI: 10.1093/gerona/glaa183

(17) Bagheri, S. H. R., Asghari, A. M., Farhadi, M., Shamshiri, A. R., Kabir, A., Kamrava, S. K., Jalessi, M., Mohebbi, A., Alizadeh, R., Honarmand, A. A., Ghalehbaghi, B., and Salimi, A. (2020) Coincidence of COVID-19 epidemic and olfactory dysfunction outbreak. *Med. J. Islam. Repub. Iran. 34*, 62.

(18) Mao, L., Jin, H., Wang, M., Hu, Y., Chen, S., He, Q., Chang, J., Hong, C., Zhou, Y., Wang, D., Miao, X., Li, Y., and Hu, B. (2020) Neurologic Manifestations of Hospitalized Patients With Coronavirus Disease 2019 in Wuhan, China. *JAMA Neurol. 77* (6), 683.

(19) Jitaroon, K., Wangworawut, Y., Ma, Y., and Patel, Z. M. (2020) Evaluation of the Incidence of Other Cranial Neuropathies in Patients With Postviral Olfactory Loss. *JAMA Otolaryngol. Head Neck Surg. 146* (5), 465.

(20) Lechien, J. R., Chiesa-Estomba, C. M., De Siati, D. R., Horoi, M., Le Bon, S. D., Rodriguez, A., Dequanter, D., Blecic, S., El Afia, F., Distinguin, L., Chekkoury-Idrissi, Y., Hans, S., Delgado, I. L., Calvo-Henriquez, C., Lavigne, P., Falanga, C., Barillari, M. R., Cammaroto, G., Khalife, M., Leich, P., Souchay, C., Rossi, C., Journe, F., Hsieh, J., Edjlali, M., Carlier, R., Ris, L., Lovato, A., De Filippis, C., Coppee, F., Fakhry, N., Ayad, T., and Saussez, S. (2020) Olfactory and gustatory dysfunctions as a clinical presentation of mild-to-moderate forms of the coronavirus disease (COVID-19): a multicenter European study. *Arch. Oto-Rhino-Laryngol. 277* (8), 2251−2261.

(21) DosSantos, M. F., Devalle, S., Aran, V., Capra, D., Roque, N. R., Coelho-Aguiar, J. d. M., Spohr, T. C. L. d. S. e., Subilhaga, J. G., Pereira, C. M., D'Andrea Meira, I., Niemeyer Soares Filho, P., and Moura-Neto, V. Neuromechanisms of SARS-CoV-2: A Review. *Front. Neuroanat.* 2020, *14* (37). DOI: 10.3389/fnana.2020.00037

(22) Giacomelli, A., Pezzati, L., Conti, F., Bernacchia, D., Siano, M., Oreni, L., Rusconi, S., Gervasoni, C., Ridolfo, A. L., Rizzardini, G., Antinori, S., and Galli, M. (2020) Self-reported olfactory and taste disorders in SARS-CoV-2 patients: a cross-sectional study. *Clin. Infect. Dis. 71* (15), 889−890.

(23) Moein, S. T., Hashemian, S. M. R., Mansourafshar, B., Khorram-Tousi, A., Tabarsi, P., and Doty, R. L. Smell dysfunction: a biomarker for COVID-19 *International Forum Allergy and Rhinol.* 2020.

(24) Baig, A. M., Khaleeq, A., Ali, U., and Syeda, H. (2020) Evidence of the COVID-19 Virus Targeting the CNS: Tissue Distribution, Host-Virus Interaction, and Proposed Neurotropic Mechanisms. *ACS Chem. Neurosci. 11* (7), 995−998.

(25) Lüers, J.-C., Klußmann, J. P., and Guntinas-Lichius, O. (2020) Die Covid-19-Pandemie und das HNO-Fachgebiet: Worauf kommt es aktuell an? *Laryngorhinootologie 99* (5), 287−291.

(26) Avula, A., Nalleballe, K., Narula, N., Sapozhnikov, S., Dandu, V., Toom, S., Glaser, A., and Elsayegh, D. (2020) COVID-19 presenting as stroke. *Brain, Behav., Immun. 87*, 115−119.

(27) Wu, Y., Xu, X., Chen, Z., Duan, J., Hashimoto, K., Yang, L., Liu, C., and Yang, C. (2020) Nervous system involvement after infection with COVID-19 and other coronaviruses. *Brain, Behav., Immun. 87*, 18−22.

(28) Carod-Artal, F. J. (2020) Neurological complications of coronavirus and COVID-19. *Rev. Neurol. 70* (9), 311−322.

(29) De Felice, F. G., Tovar-Moll, F., Moll, J., Munoz, D. P., and Ferreira, S. T. (2020) (2020) Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) and the Central Nervous System. *Trends Neurosci. 43* (6), 355−357.

(30) Pleasure, S. J., Green, A. J., and Josephson, S. A. (2020) The Spectrum of Neurologic Disease in the Severe Acute Respiratory Syndrome Coronavirus 2 Pandemic Infection: Neurologists Move to the Frontlines. *JAMA Neurol. 77* (6), 679−680.

(31) Berger, J. R. (2020) COVID-19 and the nervous system. *J. NeuroVirol. 26* (2), 143−148.

(32) Zubair, A. S., McAlpine, L. S., Gardin, T., Farhadian, S., Kuruvilla, D. E., and Spudich, S. Neuropathogenesis and Neurologic Manifestations of the Coronaviruses in the Age of Coronavirus Disease 2019: A Review *JAMA Neurol.* 2020, 77, 1018, .

(33) Montalvan, V., Lee, J., Bueso, T., De Toledo, J., and Rivas, K. (2020) Neurological manifestations of COVID-19 and other coronavirus infections: A systematic review. *Clin. Neurol. Neurosurg. 194*, 105921.

(34) Gklinos, P. Neurological manifestations of COVID-19: a review of what we know so far. *J. Neurol.* 2020, *267*, 2485,

(35) Leonardi, M., Padovani, A., and McArthur, J. C. (2020) Neurological manifestations associated with COVID-19: a review and a call for action. *J. Neurol. 267* (6), 1573−1576.

(36) Baig, A. M., and Sanders, E. C. (2020) Heralding Healthcare Professionals: Recognition of Neurological Deficits in COVID-19. *ACS Chem. Neurosci. 11* (12), 1701−1703.

(37) Das, G., Mukherjee, N., and Ghosh, S. (2020) Neurological Insights of COVID-19 Pandemic. *ACS Chem. Neurosci. 11* (9), 1206−1209.

(38) Toljan, K. (2020) Letter to the Editor Regarding the Viewpoint "Evidence of the COVID-19 Virus Targeting the CNS: Tissue Distribution, Host−Virus Interaction, and Proposed Neurotropic Mechanism. *ACS Chem. Neurosci. 11* (8), 1192−1194.

(39) Li, Y. C., Bai, W. Z., and Hashikawa, T. (2020) The neuroinvasive potential of SARS-CoV2 may play a role in the respiratory failure of COVID-19 patients. *J. Med. Virol. 92* (6), 552−555.

(40) Lochhead, J. J., and Thorne, R. G. (2012) Intranasal delivery of biologics to the central nervous system. *Adv. Drug Delivery Rev. 64* (7), 614−28.

(41) Erdö, F., Bors, L. A., Farkas, D., Bajza, Á., and Gizurarson, S. (2018) Evaluation of intranasal delivery route of drug administration for brain targeting. *Brain Res. Bull. 143*, 155−170.

(42) Gray, H. (1978) *Gray's Anatomy*, 15th ed.; Bounty Books: New York..

(43) Iwai, N., Zhou, Z., Roop, D. R., and Behringer, R. R. (2008) Horizontal basal cells are multipotent progenitors in normal and injured adult olfactory epithelium. *Stem Cells 26* (5), 1298−306.

(44) Fletcher, R. B., Das, D., Gadye, L., Street, K. N., Baudhuin, A., Wagner, A., Cole, M. B., Flores, Q., Choi, Y. G., Yosef, N., Purdom, E., Dudoit, S., Risso, D., and Ngai, J. (2017) Deconstructing Olfactory Stem Cell Trajectories at Single-Cell Resolution. *Cell Stem Cell 20* (6), 817−830.

(45) Bustamante-Marin, X. M., and Ostrowski, L. E. Cilia and Mucociliary Clearance. *Cold Spring Harbor Perspect. Biol.* 2017 *9* (4).

(46) Deprez, M., Zaragosi, L.-E., Truchi, M., Becavin, C., Ruiz Garcia, S., Arguel, M.-J., Plaisant, M., Magnone, V., Lebrigand, K., Abelanet, S., Brau, F., Paquet, A., Pe'er, D., Marquette, C.-H., Leroy, S., and Barbry, P. A single-cell atlas of the human healthy airways. *Am. J. Respir. Crit. Care Med.* 2020, DOI: 10.1164/rccm.201911-2199OC

(47) Ruiz García, S., Deprez, M., Lebrigand, K., Cavard, A., Paquet, A., Arguel, M. J., Magnone, V., Truchi, M., Caballero, I., Leroy, S., Marquette, C. H., Marcet, B., Barbry, P., and Zaragosi, L. E. Novel dynamics of human mucociliary differentiation revealed by single-cell RNA sequencing of nasal epithelial cultures *Development* 2019, dev.177428,

(48) Ou, X., Liu, Y., Lei, X., Li, P., Mi, D., Ren, L., Guo, L., Guo, R., Chen, T., Hu, J., Xiang, Z., Mu, Z., Chen, X., Chen, J., Hu, K., Jin, Q., Wang, J., and Qian, Z. (2020) Characterization of spike glycoprotein of SARS-CoV-2 on virus entry and its immune cross-reactivity with SARS-CoV. *Nat. Commun. 11* (1), 1620.

(49) Olender, T., Keydar, I., Pinto, J. M., Tatarskyy, P., Alkelai, A., Chien, M. S., Fishilevich, S., Restrepo, D., Matsunami, H., Gilad, Y., and Lancet, D. (2016) The human olfactory transcriptome. *BMC Genomics* 17 (1), 619.

(50) Dalton, P. (2004) Olfaction and anosmia in rhinosinusitis. *Curr. Allergy Asthma Rep.* 4 (3), 230−236.

(51) Akerlund, A., Bende, M., and Murphy, C. (1995) Olfactory threshold and nasal mucosal changes in experimentally induced common cold. *Acta Oto-Laryngol.* 115 (1), 88−92.

(52) Yamagishi, M., and Nakano, Y. (1992) A re-evaluation of the classification of olfactory epithelia in patients with olfactory disorders. *Eur. Arch. Oto-Rhino-Laryngol.* 249 (7), 393−399.

(53) Suzuki, M., Saito, K., Min, W. P., Vladau, C., Toida, K., Itoh, H., and Murakami, S. (2007) Identification of viruses in patients with postviral olfactory dysfunction. *Laryngoscope* 117 (2), 272−277.

(54) van Riel, D., Verdijk, R., and Kuiken, T. (2015) The olfactory nerve: a shortcut for influenza and other viral diseases into the central nervous system. *Journal of pathology* 235 (2), 277−287.

(55) Hamming, I., Timens, W., Bulthuis, M. L., Lely, A. T., Navis, G., and van Goor, H. (2004) Tissue distribution of ACE2 protein, the functional receptor for SARS coronavirus. A first step in understanding SARS pathogenesis. *J. Pathol.* 203 (2), 631−637.

(56) Xu, H., Zhong, L., Deng, J., Peng, J., Dan, H., Zeng, X., Li, T., and Chen, Q. (2020) High expression of ACE2 receptor of 2019-nCoV on the epithelial cells of oral mucosa. *Int. J. Oral Sci.* 12 (1), 8.

(57) Hikmet, F., Méar, L., Edvinsson, Å., Micke, P., Uhlén, M., and Lindskog, C. (2020) The protein expression profile of ACE2 in human tissues. *Mol. Syst. Biol.* 16 (7), No. e9610.

(58) Wang, K., Chen, W., Zhou, Y.-S., Lian, J.-Q., Zhang, Z., Du, P., Gong, L., Zhang, Y., Cui, H.-Y., Geng, J.-J., Wang, B., Sun, X.-X., Wang, C.-F., Yang, X., Lin, P., Deng, Y.-Q., Wei, D., Yang, X.-M., Zhu, Y.-M., Zhang, K., Zheng, Z.-H., Miao, J.-L., Guo, T., Shi, Y., Zhang, J., Fu, L., Wang, Q.-Y., Bian, H., Zhu, P., and Chen, Z.-N. SARS-CoV-2 invades host cells via a novel route: CD147-spike protein *biorXiv* 2020, https://www.biorxiv.org/content/biorxiv/early/2020/03/14/2020.03.14.988345.full.pdf. (accessed 2020-Mar-14).

(59) Zang, R., Castro, M. F. G., McCune, B. T., Zeng, Q., Rothlauf, P. W., Sonnek, N. M., Liu, Z., Brulois, K. F., Wang, X., Greenberg, H. B., Diamond, M. S., Ciorba, M. A., Whelan, S. P. J., and Ding, S. (2020) TMPRSS2 and TMPRSS4 promote SARS-CoV-2 infection of human small intestinal enterocytes. *Sci. Immunol.* 5 (47), eabc3582.

(60) Mygind, N., and Anggård, A. (1984) Anatomy and physiology of the nose–pathophysiologic alterations in allergic rhinitis. *Clin. Rev. Allergy* 2 (3), 173−88.

(61) Collins, L. M., and Dawes, C. (1987) The surface area of the adult human mouth and thickness of the salivary film covering the teeth and oral mucosa. *J. Dent. Res.* 66 (8), 1300−1302.

(62) Butowt, R., and Bilinska, K. (2020) SARS-CoV-2: Olfaction, Brain Infection, and the Urgent Need for Clinical Samples Allowing Earlier Virus Detection. *ACS Chem. Neurosci.* 11 (9), 1200−1203.

(63) Bilinska, K., Jakubowska, P., Von Bartheld, C. S., and Butowt, R. (2020) Expression of the SARS-CoV-2 Entry Proteins, ACE2 and TMPRSS2, in Cells of the Olfactory Epithelium: Identification of Cell Types and Trends with Age. *ACS Chem. Neurosci.* 11 (11), 1555−1562.

(64) Bryche, B., St Albin, A., Murri, S., Lacôte, S., Pulido, C., Ar Gouilh, M., Lesellier, S., Servat, A., Wasniewski, M., Picard-Meyer, E., Monchatre-Leroy, E., Volmer, R., Rampin, O., Le Goffic, R., Marianneau, P., and Meunier, N., Massive transient damage of the olfactory epithelium associated with infection of sustentacular cells by SARS-CoV-2 in golden Syrian hamsters. *Brain, Behav., Immun.* 2020, S0889-1591(20)31358-1. DOI: 10.1016/j.bbi.2020.06.032

(65) Yuan, Y., Cao, D., Zhang, Y., Ma, J., Qi, J., Wang, Q., Lu, G., Wu, Y., Yan, J., Shi, Y., Zhang, X., and Gao, G. F. (2017) Cryo-EM structures of MERS-CoV and SARS-CoV spike glycoproteins reveal the dynamic receptor binding domains. *Nat. Commun.* 8, 15092.

(66) Kanageswaran, N., Demond, M., Nagel, M., Schreiner, B. S., Baumgart, S., Scholz, P., Altmüller, J., Becker, C., Doerner, J. F., Conrad, H., Oberland, S., Wetzel, C. H., Neuhaus, E. M., Hatt, H., and Gisselmann, G. (2015) Deep sequencing of the murine olfactory receptor neuron transcriptome. *PLoS One* 10 (1), No. e0113170.

(67) Saraiva, L. R., Ibarra-Soria, X., Khan, M., Omura, M., Scialdone, A., Mombaerts, P., Marioni, J. C., and Logan, D. W. (2015) Hierarchical deconstruction of mouse olfactory sensory neurons: from whole mucosa to single-cell RNA-seq. *Sci. Rep.* 5, 18178.

(68) Sungnak, W., Huang, N., Bécavin, C., Berg, M., Queen, R., Litvinukova, M., Talavera-López, C., Maatz, H., Reichart, D., Sampaziotis, F., Worlock, K. B., Yoshida, M., and Barnes, J. L. (2020) SARS-CoV-2 entry factors are highly expressed in nasal epithelial cells together with innate immune genes. *Nat. Med.* 26 (5), 681−687.

(69) Brann, D., Tsukahara, T., Weinreb, C., Lipovsek, M., Van den Berge, K., Gong, B., Chance, R., Macaulay, I. C., Chou, H.-j., Fletcher, R., Das, D., Street, K., Roux de Bezieux, H., Choi, Y.-G., Risso, D., Dudoit, S., Purdom, E., Mill, J. C., Hachem, R. A., Matsunami, H., Logan, D. W., Goldstein, B., Grubb, M. S., Ngai, J., and Datta, S. R. Non-neuronal expression of SARS-CoV-2 entry genes in the olfactory system suggests mechanisms underlying COVID-19-associated anosmia *biorXiv* 2020, 2020.03.25.009084. https://www.biorxiv.org/content/biorxiv/early/2020/04/09/2020.03.25.009084.full.pdf (accessed 2020-04-10).

(70) Chen, R., Wang, K., Yu, J., Howard, D., French, L., Chen, Z., Wen, C., and Xu, Z. The spatial and cell-type distribution of SARS-CoV-2 receptor ACE2 in human and mouse brain *biorXiv* 2020, 2020.04.07.030650. https://www.biorxiv.org/content/biorxiv/early/2020/05/19/2020.04.07.030650.full.pdf. (accessed 2020-May-19).

(71) Lamers, M. M., Beumer, J., van der Vaart, J., Knoops, K., Puschhof, J., Breugem, T. I., Ravelli, R. B. G., van Schayck, J. P., Mykytyn, A. Z., Duimel, H. Q., van Donselaar, E., Riesebosch, S., Kuijpers, H. J. H., Schipper, D., van de Wetering, W. J., de Graaf, M., Koopmans, M., Cuppen, E., Peters, P. J., Haagmans, B. L., and Clevers, H. (2020) SARS-CoV-2 Productively Infects Human Gut Enterocytes. *Science* 369 (6499), 50−54.

(72) Ziegler, C. G. K., Allon, S. J., Nyquist, S. K., Mbano, I. M., Miao, V. N., Tzouanas, C. N., Cao, Y., Yousif, A. S., Bals, J., Hauser, B. M., Feldman, J., Muus, C., Wadsworth, M. H., 2nd, Kazer, S. W., Hughes, T. K., Doran, B., Gatter, G. J., Vukovic, M., Taliaferro, F., Mead, B. E., Guo, Z., Wang, J. P., Gras, D., Plaisant, M., Ansari, M., Angelidis, I., Adler, H., Sucre, J. M. S., Taylor, C. J., Lin, B., Waghray, A., Mitsialis, V., Dwyer, D. F., Buchheit, K. M., Boyce, J. A., Barrett, N. A., Laidlaw, T. M., Carroll, S. L., Colonna, L., Tkachev, V., Peterson, C. W., Yu, A., Zheng, H. B., Gideon, H. P., Winchell, C. G., Lin, P. L., Bingle, C. D., Snapper, S. B., Kropski, J. A., Theis, F. J., Schiller, H. B., Zaragosi, L. E., Barbry, P., Leslie, A., Kiem, H. P., Flynn, J. L., Fortune, S. M., Berger, B., Finberg, R. W., Kean, L. S., Garber, M., Schmidt, A. G., Lingwood, D., Shalek, A. K., Ordovas-Montanes, J., et al. (2020) SARS-CoV-2 Receptor ACE2 Is an Interferon-Stimulated Gene in Human Airway Epithelial Cells and Is Detected in Specific Cell Subsets across Tissues. *Cell* 181 (5), 1016−1035.

(73) Fodoulian, L., Tuberosa, J., Rossier, D., Boillat, M., Kan, C.-D., Pauli, V., Egervari, K., Lobrinus, J. A., Landis, B., Carleton, A., and Rodriguez, I. SARS-CoV-2 receptor and entry genes are expressed by sustentacular cells in the human olfactory neuroepithelium *biorXiv* 2020, 2020.03.31.013268. https://www.biorxiv.org/content/biorxiv/early/2020/05/30/2020.03.31.013268.full.pdf. (accessed 2020-Mar-31).

(74) Ali, F., Elserafy, M., Alkordi, M., and Amin, M. ACE2 coding variants in different populations and their potential impact on SARS-CoV-2 binding affinity *biorXiv* 2020, 2020.05.08.084384. https://www.biorxiv.org/content/biorxiv/early/2020/05/08/2020.05.08.084384.full.pdf. (accessed 2020-May-10).

(75) Muus, C., Luecken, M. D., Eraslan, G., Waghray, A., Heimberg, G., Sikkema, L., Kobayashi, Y., Vaishnav, E. D., Subramanian, A., Smilie, C., Jagadeesh, K., Duong, E. T., Fiskin, E., Triglia, E. T., Ansari, M., Cai, P., Lin, B., Buchanan, J., Chen, S., Shu, J., Haber, A. L., Chung, H., Montoro, D. T., Adams, T., Aliee, H., Samuel, J., Andrusivova, A. Z., Angelidis, I., Ashenberg, O., Bassler, K., Bécavin, C., Benhar, I., Bergenstråhle, J., Bergenstråhle, L., Bolt, L., Braun, E., Bui, L. T., Chaffin, M., Chichelnitskiy, E., Chiou, J., Conlon, T. M., Cuoco, M. S.,

Deprez, M., Fischer, D. S., Gillich, A., Gould, J., Guo, M., Gutierrez, A. J., Habermann, A. C., Harvey, T., He, P., Hou, X., Hu, L., Jaiswal, A., Jiang, P., Kapellos, T., Kuo, C. S., Larsson, L., Kyungtae Lim, M. A. L.-G., Litvinuková, M., Lu, J., Maatz, H., Madissoon, E., Mamanova, L., Manakongtreecheep, K., Marquette, C.-H., Mbano, I., McAdams, A. M., Metzger, R. J., Nabhan, A. N., Nyquist, S. K., Ordovas-Montanes, J., Penland, L., Poirion, O. B., Poli, S., Qi, C., Reichart, D., Rosas, I., Schupp, J., Sinha, R., Sit, R. V., Slowikowski, K., Slyper, M., Smith, N., Sountoulidis, A., Strunz, M., Sun, D., Talavera-López, C., Tan, P., Tantivit, J., Travaglini, K. J., Tucker, N. R., Vernon, K., Wadsworth, M. H., Waldmann, J., Wang, X., Yan, W., Zhao, W., and Ziegler, C. G. K. Integrated analyses of single-cell atlases reveal age, gender, and smoking status associations with cell type-specific expression of mediators of SARS-CoV-2 viral entry and highlights inflammatory programs in putative target cells. *biorXiv* 2020, 2020.04.19.049254. https://www.biorxiv.org/content/biorxiv/early/2020/04/20/2020.04.19.049254.full.pdf (accessed 2020-May-22).

(76) Sajuthi, S. P., DeFord, P., Jackson, N. D., Montgomery, M. T., Everman, J. L., Rios, C. L., Pruesse, E., Nolin, J. D., Plender, E. G., Wechsler, M. E., Mak, A. C., Eng, C., Salazar, S., Medina, V., Wohlford, E. M., Huntsman, S., Nickerson, D. A., Germer, S., Zody, M. C., Abecasis, G., Kang, H. M., Rice, K. M., Kumar, R., Oh, S., Rodriguez-Santana, J., Burchard, E. G., and Seibold, M. A. Type 2 and interferon inflammation strongly regulate SARS-CoV-2 related gene expression in the airway epithelium *biorXiv* 2020, 2020.04.09.034454. https://www.biorxiv.org/content/biorxiv/early/2020/04/10/2020.04.09.034454.full.pdf. (accessed 2020.04.10).

(77) Kimura, H., Francisco, D., Conway, M., Martinez, F. D., Vercelli, D., Polverino, F., Billheimer, D., and Kraft, M. (2020) Type 2 Inflammation Modulates ACE2 and TMPRSS2 in Airway Epithelial Cells. *J. Allergy Clin. Immunol.* 146 (1), 80−88. e8

(78) Lee, I. T., Nakayama, T., Wu, C.-T., Goltsev, Y., Jiang, S., Gall, P. A., Liao, C.-K., Shih, L.-C., Schurch, C. M., McIlwain, D. R., Chu, P., Borchard, N. A., Zarabanda, D., Dholakia, S. S., Yang, A., Kim, D., Kanie, T., Lin, C.-D., Tsai, M.-H., Phillips, K. M., Kim, R., Overdevest, J. B., Tyler, M. A., Yan, C. H., Lin, C.-F., Lin, Y.-T., Bau, D.-T., Tsay, G. J., Patel, Z. M., Tsou, Y.-A., Tai, C.-J., Yeh, T.-H., Hwang, P. H., Nolan, G. P., Nayak, J. V., and Jackson, P. K., Robust ACE2 protein expression localizes to the motile cilia of the respiratory tract epithelia and is not increased by ACE inhibitors or angiotensin receptor blockers. *medRxiv* 2020, 2020.05.08.20092866. DOI: 10.1101/2020.05.08.20092866 (accessed 2020-07-22).

(79) Leopold, P. L., O'Mahony, M. J., Lian, X. J., Tilley, A. E., Harvey, B.-G., and Crystal, R. G. (2009) Smoking is associated with shortened airway cilia. *PLoS One* 4 (12), e8157−e8157.

(80) Hou, Y. J., Okuda, K., Edwards, C. E., Martinez, D. R., Asakura, T., Dinnon, K. H., III, Kato, T., Lee, R. E., Yount, B. L., Mascenik, T. M., Chen, G., Olivier, K. N., Ghio, A., Tse, L. V., Leist, S. R., Gralinski, L. E., Schäfer, A., Dang, H., Gilmore, R., Nakano, S., Sun, L., Fulcher, M. L., Livraghi-Butrico, A., Nicely, N. I., Cameron, M., Cameron, C., Kelvin, D. J., de Silva, A., Margolis, D. M., Markmann, A., Bartelt, L., Zumwalt, R., Martinez, F. J., Salvatore, S. P., Borczuk, A., Tata, P. R., Sontake, V., Kimple, A., Jaspers, I., O'Neal, W. K., Randell, S. H., Boucher, R. C., and Baric, R. S. (2020) SARS-CoV-2 Reverse Genetics Reveals a Variable Infection Gradient in the Respiratory Tract. *Cell* 182 (2), 429−446.

(81) Smith, J. C., Sausville, E. L., Girish, V., Yuan, M. L., Vasudevan, A., John, K. M., and Sheltzer, J. M. (2020) Cigarette Smoke Exposure and Inflammatory Signaling Increase the Expression of the SARS-CoV-2 Receptor ACE2 in the Respiratory Tract. *Dev. Cell* 53 (5), 514−529.

(82) Xia, H., and Lazartigues, E. (2008) Angiotensin-converting enzyme 2 in the brain: properties and future directions. *J. Neurochem.* 107 (6), 1482−1484.

(83) Xu, P., Sriramula, S., and Lazartigues, E. (2011) ACE2/ANG-(1−7)/Mas pathway in the brain: the axis of good. *Am. J. Physiol.* 300 (4), R804−817.

(84) Garcia-Garrote, M., Perez-Villalba, A., Garrido-Gil, P., Belenguer, G., Parga, J. A., Perez-Sanchez, F., Labandeira-Garcia, J. L., Fariñas, I., and Rodriguez-Pallares, J. (2019) Interaction between Angiotensin

Type 1, Type 2, and Mas Receptors to Regulate Adult Neurogenesis in the Brain Ventricular-Subventricular Zone. *Cells* 8 (12), 1551.

(85) Chappell, M. C., Brosnihan, K. B., Diz, D. I., and Ferrario, C. M. (1989) Identification of angiotensin-(1−7) in rat brain. Evidence for differential processing of angiotensin peptides. *J. Biol. Chem.* 264 (28), 16518−16523.

(86) Rocha, N. P., Simoes, E. S. A. C., Prestes, T. R. R., Feracin, V., Machado, C. A., Ferreira, R. N., Teixeira, A. L., and de Miranda, A. S. (2018) RAS in the Central Nervous System: Potential Role in Neuropsychiatric Disorders. *Curr. Med. Chem.* 25 (28), 3333−3352.

(87) Doobay, M. F., Talman, L. S., Obr, T. D., Tian, X., Davisson, R. L., and Lazartigues, E. (2007) Differential expression of neuronal ACE2 in transgenic mice with overexpression of the brain renin-angiotensin system. *Am. J. Physiol.* 292 (1), R373−81.

(88) Marini, J. J., and Gattinoni, L. Management of COVID-19 Respiratory Distress. *JAMA* 2020, 323, 2329, .

(89) Marini, J. J. Dealing with the CARDS of COVID-19. *Crit. Care Med.* 2020, 48 (8), 1239−1241.

(90) Meinhardt, J., Radke, J., Dittmayer, C., Mothes, R., Franz, J., Laue, M., Schneider, J., Bruenink, S., Hassan, O., Stenzel, W., Windgassen, Marc, Roessler, L., Goebel, H.-H., Martin, H., Nitsche, A., Schulz-Schaeffer, W., Hakroush, S., Winkler, M. S., Tampe, B., Elezkurtaj, S., Horst, D., Oesterhelweg, L., Tsokos, M., Ingold Heppner, B., Stadelmann, C., Drosten, C., Corman, V. M., Radbruch, H., and Heppner, F. L. Olfactory transmucosal SARS-CoV-2 invasion as port of Central Nervous System entry in COVID-19 patients *biorXiv* 2020, 2020.06.04.135012. https://www.biorxiv.org/content/biorxiv/early/2020/06/04/2020.06.04.135012.full.pdf (accessed 2020-May-22).

(91) Filatov, A., Sharma, P., Hindi, F., and Espinosa, P. S. (2020) Neurological Complications of Coronavirus Disease (COVID-19): Encephalopathy. *Cureus* 12 (3), No. e7352.

(92) Espinosa, P. S, Rizvi, Z., Sharma, P., Hindi, F., and Filatov, A. (2020) Neurological Complications of Coronavirus Disease (COVID-19): Encephalopathy, MRI Brain and Cerebrospinal Fluid Findings: Case 2. *Cureus* 12 (5), No. e7930.

(93) Ye, M., Ren, Y., and Lv, T. (2020) Encephalitis as a clinical manifestation of COVID-19. *Brain, Behav., Immun.* 88 (10), 945−946.

(94) Nash, T. C., and Buchmeier, M. J. (1997) Entry of mouse hepatitis virus into cells by endosomal and nonendosomal pathways. *Virology* 233 (1), 1−8.

(95) Pu, Y., and Zhang, X. (2008) Mouse hepatitis virus type 2 enters cells through a clathrin-mediated endocytic pathway independent of Eps15. *J. Virol.* 82 (16), 8112−23.

(96) Pober, J. S., and Sessa, W. C. (2007) Evolving functions of endothelial cells in inflammation. *Nat. Rev. Immunol.* 7 (10), 803−15.

(97) Bryce, C., Grimes, Z., Pujadas, E., Ahuja, S., Beasley, M. B., Albrecht, R., Hernandez, T., Stock, A., Zhao, Z., Al Rasheed, M., Chen, J., Li, L., Wang, D., Corben, A., Haines, K., Westra, W., Umphlett, M., Gordon, R. E., Reidy, J., Petersen, B., Salem, F., Fiel, M., El Jamal, S. M., Tsankova, N. M., Houldsworth, J., Mussa, Z., Liu, W.-C., Veremis, B., Sordillo, E., Gitman, M., Nowak, M., Brody, R., Harpaz, N., Merad, M., Gnjatic, S., Donnelly, R., Seigler, P., Keys, C., Cameron, J., Moultrie, I., Washington, K.-L., Treatman, J., Sebra, R., Jhang, J., Firpo, A., Lednicky, J., Paniz-Mondolfi, A., Cordon-Cardo, C., and Fowkes, M. Pathophysiology of SARS-CoV-2: targeting of endothelial cells renders a complex disease with thrombotic microangiopathy and aberrant immune response. The Mount Sinai COVID-19 autopsy experience. *medRxiv* 2020, 2020.05.18.20099960. https://www.medrxiv.org/content/medrxiv/early/2020/05/22/2020.05.18.20099960.full.pdf. (accessed 2020-06-12).

(98) Mehta, P., McAuley, D. F., Brown, M., Sanchez, E., Tattersall, R. S., and Manson, J. J. (2020) COVID-19: consider cytokine storm syndromes and immunosuppression. *Lancet* 395 (10229), 1033−1034.

(99) Santiago-Tirado, F. H., and Doering, T. L. (2017) False friends: Phagocytes as Trojan horses in microbial brain infections. *PLoS Pathog.* 13 (12), e1006680−e1006680.

(100) Louveau, A., Herz, J., and Alme, M. N. (2018) CNS lymphatic drainage and neuroinflammation are regulated by meningeal lymphatic vasculature. *Nat. Neurosci.* 21 (10), 1380.

(101) Desforges, M., Le Coupanec, A., Dubeau, P., Bourgouin, A., Lajoie, L., Dubé, M., and Talbot, P. J. (2020) Human Coronaviruses and Other Respiratory Viruses: Underestimated Opportunistic Pathogens of the Central Nervous System? *Viruses 12* (1), 14.

(102) Dubé, M., Le Coupanec, A., Wong, A. H. M., Rini, J. M., Desforges, M., and Talbot, P. J. (2018) Axonal Transport Enables Neuron-to-Neuron Propagation of Human Coronavirus OC43. *J. Virol. 92* (17), e00404−18.

(103) Gershon, A. A., and Gershon, M. D. (2013) Pathogenesis and Current Approaches to Control of Varicella-Zoster Virus Infections. *Clin. Microbiol. Res. 26* (4), 728−743.

(104) Ray, P., Torck, A., Quigley, L., Wangzhou, A., Neiman, M., Rao, C., Lam, T., Kim, J. Y., Kim, T. H., Zhang, M. Q., Dussor, G., and Price, T. J. (2018) Comparative transcriptome profiling of the human and mouse dorsal root ganglia: an RNA-seq-based resource for pain and sensory neuroscience research. *Pain 159* (7), 1325−1345.

(105) Shiers, S., Ray, P., Wangzhou, A., Esteves Tatsui, C., Rhines, L., Li, Y., Uhelski, M. L., Dougherty, P. M., and Price, T. J.ACE2 expression in human dorsal root ganglion sensory neurons: implications for SARS-CoV-2 virus-induced neurological effects *biorXiv* 2020, 2020.05.28.122374. https://www.biorxiv.org/content/biorxiv/early/2020/05/29/2020.05.28.122374.full.pdf (accessed 2020-05-22).

(106) Hockley, J. R. F., Taylor, T. S., Callejo, G., Wilbrey, A. L., Gutteridge, A., Bach, K., Winchester, W. J., Bulmer, D. C., McMurray, G., and Smith, E. S. J. (2019) Single-cell RNAseq reveals seven classes of colonic sensory neuron. *Gut 68* (4), 633−644.

(107) von Buchholtz, L. J., Lam, R. M., Emrick, J. J., Chesler, A. T., and Ryba, N. J. P. Assigning transcriptomic class in the trigeminal ganglion using multiplex in situ hybridization and machine learning *Pain* 2020, DOI: 10.1097/j.pain.0000000000001911

(108) Barragán-Iglesias, P., Franco-Enzástiga, Ú., Jeevakumar, V., Shiers, S., Wangzhou, A., Granados-Soto, V., Campbell, Z. T., Dussor, G., and Price, T. J. (2020) Type I Interferons Act Directly on Nociceptors to Produce Pain Sensitization: Implications for Viral Infection-Induced Pain. *J. Neurosci. 40* (18), 3517−3532.

(109) Harahsheh, A. S., Dahdah, N., Newburger, J. W., Portman, M. A., Piram, M., Tulloh, R., McCrindle, B. W., de Ferranti, S. D., Cimaz, R., Truong, D. T., and Burns, J. C. (2020) Missed or Delayed Diagnosis of Kawasaki Disease During the 2019 Novel Coronavirus Disease (COVID-19) Pandemic. *J. Pediatr. 222*, 261−262.

(110) Verdoni, L., Mazza, A., Gervasoni, A., Martelli, L., Ruggeri, M., Ciuffreda, M., Bonanomi, E., and D'Antiga, L. (2020) An outbreak of severe Kawasaki-like disease at the Italian epicentre of the SARS-CoV-2 epidemic: an observational cohort study. *Lancet 395* (10239), 1771−1778.

(111) Sachdeva, M., Gianotti, R., Shah, M., Bradanini, L., Tosi, D., Veraldi, S., Ziv, M., Leshem, E., and Dodiuk-Gad, R. P. (2020) Cutaneous manifestations of COVID-19: Report of three cases and a review of literature. *J. Dermatol. Sci. 98* (2), 75−81.