

Anosmia in COVID-19: Underlying Mechanisms and Assessment of an Olfactory Route to Brain Infection

The Neuroscientist
1–22
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DOI: 10.1177/1073858420956905
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Abstract

In recent months it has emerged that the novel coronavirus—responsible for the COVID-19 pandemic—causes reduction of smell and taste in a large fraction of patients. The chemosensory deficits are often the earliest, and sometimes the only signs in otherwise asymptomatic carriers of the SARS-CoV-2 virus. The reasons for the surprisingly early and specific chemosensory dysfunction in COVID-19 are now beginning to be elucidated. In this hypothesis review, we discuss implications of the recent finding that the prevalence of smell and taste dysfunction in COVID-19 patients differs between populations, possibly because of differences in the spike protein of different virus strains or because of differences in the host proteins that enable virus entry, thus modifying infectivity. We review recent progress in defining underlying cellular and molecular mechanisms of the virus-induced anosmia, with a focus on the emerging crucial role of sustentacular cells in the olfactory epithelium. We critically examine the current evidence whether and how the SARS-CoV-2 virus can follow a route from the olfactory epithelium in the nose to the brain to achieve brain infection, and we discuss the prospects for using the smell and taste dysfunctions seen in COVID-19 as an early and rapid diagnostic screening tool.

Keywords

anosmia, COVID-19, olfactory epithelium, SARS-CoV-2, ACE2, prevalence, diagnosis, hyposmia, smell loss, taste, brain infection

Introduction

Reduction of smell and taste is now recognized as one of the cardinal symptoms of COVID-19. The deficit appears to be most often transient, with a regaining of smell and taste after several days to weeks, but the anosmia differs from other virus-associated deficits in its sudden onset and its rapid recovery. Multiple reviews have covered this topic—so why is the current review needed? We have come to realize that to understand COVID-19, it is necessary to consider multiple dimensions, from the cellular-molecular level to psychophysical and clinical features, as well as genetics and epidemiology. Relating different aspects of the disease in a holistic approach has been lacking in previous reviews; we will show that taking into account and integrating multiple disciplines provides a more complete insight and synthesis.

Anosmia and hypogeusia were not initially recognized to be linked to COVID-19; they were mentioned to affect only about 5% of COVID-19 patients in one of the first studies from China (Mao and others 2020), but a much higher prevalence was reported in subsequent studies from Europe, the Middle East, and North America (Agyeman and others 2020; Hannum and others 2020;

Passarelli and others 2020; Printza and Constantinidis 2020; Sedaghat and others 2020; Tong and others 2020; von Bartheld and others 2020). Why is the reduction in smell and taste one of the first symptoms of COVID-19, and why were these deficits recognized to be a cardinal symptom of COVID-19 only when the pandemic had moved beyond East Asia? We discuss possible explanations for early symptoms and for the population differences and their implications. Key to understanding such differences in infectivity of SARS-CoV-2 may lie in the frequency of variants in the virus entry proteins, ACE2

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and TMPRSS2, which may depend on cell type and population, with implications for infectivity, virus spread, and therefore managing the COVID-19 pandemic.

It has been a major mystery how the virus affects the senses of smell and taste. Significant progress has now been made to begin to elucidate the cellular and molecular mechanisms of coronavirus-induced anosmia. Recent work has provided new insights into the cell types in the olfactory epithelium that express the relevant virus entry proteins (Bilinska and others 2020) and that accumulate the virus after infection (Bryche and others 2020). Much less is known about the underlying mechanisms that may explain taste reduction in COVID-19. ACE2 is expressed in epithelial cells of the tongue (Sato and others 2020; Vaira and others 2020c; Xu and others 2020; Cooper and others 2020), but probably not in taste buds (Wang and others 2020e), yet ACE2 inhibitor drugs are known to induce taste (and smell) disorders (Irvin and Viau 1986; Naik and others 2010; Bertlich and others 2020). In the olfactory epithelium, the evidence suggests a distinct cascade of cellular events that can explain the transient anosmia in COVID-19. Whether and how the SARS-CoV-2 virus may utilize a route from the nose to infect the brain has been and still is a question of major interest and concern. We review a series of relevant studies that provide deeper insights on this topic, and we propose new hypotheses of how SARS-CoV-2 may gain access to the brain without relying on transport within olfactory neurons. In this context, we explain the importance of developing and investigating new transgenic mouse models for future research in this field. We also review the prospects for making use of the anosmia seen in COVID-19 as an early, rapid, and surprisingly effective diagnostic screening tool.

What Is the Prevalence of Smell and Taste Dysfunction in COVID-19 Patients?

The literature on the prevalence of chemosensory dysfunctions in COVID-19 initially appeared to be confusing: wide ranges of prevalence were reported by different studies. In the first two months of the COVID-19 pandemic, clinicians considered such deficits to be a rare occurrence (Chen and others 2020a; Guan and others 2020; Mao and others 2020; Wang and others 2020d; reviewed by da Costa and others 2020). The first report that recognized smell and taste reduction to be a much more prevalent symptom came out of Germany (Streck 2020), and subsequent studies have confirmed that a high prevalence of approximately 60% is the norm, especially outside of East Asia (von Bartheld and others 2020). Several reviews have summarized findings of the early studies on this topic (Agyeman and others 2020; Hannum and others 2020; Passarelli and others 2020; Printza and

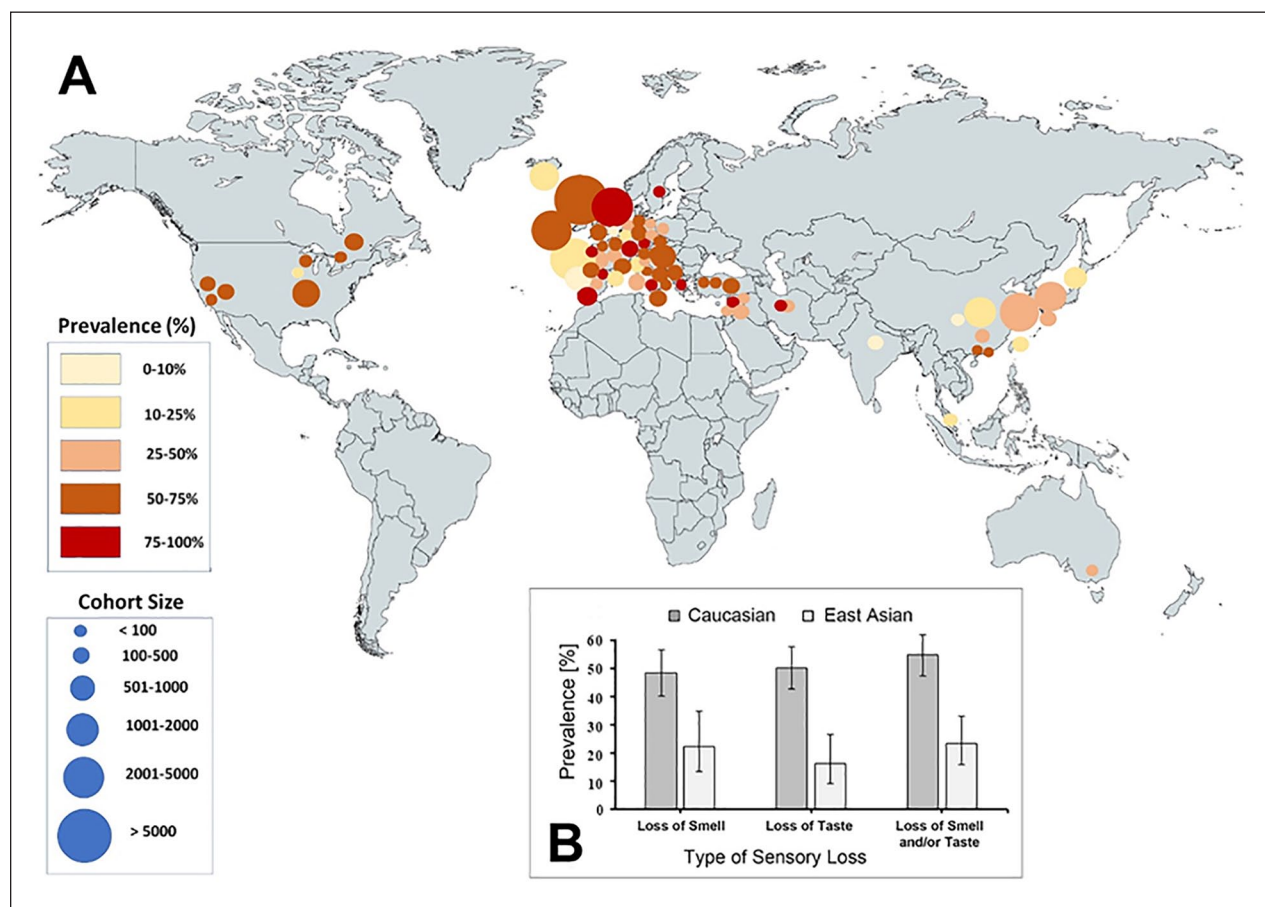
Constantinidis 2020; Sedaghat and others 2020; Tong and others 2020), which were followed by a more comprehensive review and meta-analysis (von Bartheld and others 2020). Worldwide, the prevalence of olfactory deficits in COVID-19 patients was calculated to be 44.1%, the prevalence of taste deficits was 43.3%, and the prevalence for any chemosensory deficits was 49.0% (Table 1). The prevalence of anosmia/ageusia in COVID-19 is generally thought to be an underestimate (Vaira and others 2020b; Tong and others 2020; von Bartheld and others 2020), because most studies rely on the patient telling the researcher about their subjective impressions, although some studies report that the results of subjective and objective measures are roughly equivalent (Parma and others 2020). Several researchers have noticed a possible difference in the prevalence of chemosensory deficits between populations in East Asia and in Western countries (Dell’Era and others 2020; Lovato and others 2020; Lechien and others 2020a; Meng and others 2020; Qiu and others 2020). Intriguingly, the most recent and comprehensive systematic review, of 30,264 patients, demonstrated a significant, nearly 3-fold higher prevalence in olfaction and/or taste impairment in populations in Western countries than in populations in East Asia, and the difference appears to be independent of age or disease severity (von Bartheld and others 2020, Fig. 1A and B). There are no data yet specifically on populations in Africa, South America, or South Asia in this respect. The nasal epithelium has been shown to have a larger viral load than the epithelium in the lower respiratory tract (Hou and others 2020; Meinhardt and others 2020; Rockx and others 2020; Wang and others 2020c; Zou and others 2020). Therefore, population differences in viral load could have far-reaching implications for infectivity and virus spreading, and ultimately for successful management of the pandemic.

Why Is There a Difference in Prevalence of Chemosensory Deficits between Populations in East Asia Compared with Those in Western Countries?

One “trivial” explanation that needs to be considered is that the smell and taste dysfunctions in East Asia were underreported, possibly because these symptoms were overlooked in China, when early in the pandemic anosmia did not yet receive much publicity. However, more recent studies, including those from Korea, Singapore, and Japan, also report much lower prevalence than studies from Western countries (von Bartheld and others 2020; Fig. 1A and B), so underreporting alone is unlikely to explain the difference between populations. Two other reasons may account for the different rates of smell dysfunction among COVID-19

Table 1. Prevalence of Dysfunctions in Smell, Taste, and Any Chemosensory Perception in COVID-19 Patients According to Our Recent Review and Meta-Analysis (von Bartheld and others 2020).

Sensory Deficit	Population	Cohort Number	Prevalence (%)
Smell	East Asia	4587	22.4
	Western countries	13,897	48.4
	All	18,484	44.1
Taste	East Asia	5747	16.2
	Western countries	10,168	50.3
	All	15,915	43.3
Smell and/or taste	East Asia	8253	23.4
	Western countries	22,011	54.7
	All	30,264	49.0

**Figure 1.** Prevalence of chemosensory deficits in COVID-19 patients. (A) World map based on 68 studies with a total of 30,264 patients (updated version, original from: von Bartheld and others 2020). (B) Prevalence of chemosensory dysfunction in COVID-19 patients in Western countries and East Asia according to a recent meta-analysis (von Bartheld and others 2020). Error bars are 95% confidence intervals. Data are based on a total of 22,011 Caucasian and 8253 East Asian patients with COVID-19 from $n = 61$ cohorts and $n = 12$ cohorts, respectively.

patients in different populations: genetic variation at the level of the virus, or genetic variation at the level of the host. Variants of SARS-CoV-2 may differ in geography, for

example, due to mutations in the spike protein (Grubaugh and others 2020; Korber and others 2020; Li and others 2020a; Phelan and others 2020; Zhang and others 2020);

“Type C” is predominant in East Asia, while both “Type C” and “Type A” occur outside of East Asia (Forster and others 2020). Alternatively, or in addition, there may be genetic polymorphism in the ACE2 or TMPRSS2 host receptors.

The receptor binding domain (RBD) of the virus’ spike protein (subunit S1) binds with high affinity to the peptidase domain of the entry protein ACE2 and thereby determines viral tropism and infectivity (Shang and others 2020). Therefore, genetic variability and the mutation rate within the RBD domain is of particular interest in the context of population differences in the prevalence of anosmia and ageusia. Recent studies indicate that SARS-CoV-2 has a significantly lower mutation rate as compared to SARS-CoV-1 virus and that the critical RBD domain of the spike glycoprotein is particularly well conserved (Jia and others 2020). Even though some genetic variability and mutation hot spots were identified in the RBD, which may affect its binding to the ACE2 receptor (Jia and others 2020; Ou and others 2020), these mutations were not restricted to one specific geographic area, but were present in Europe, Asia, and America (Ou and others 2020; van Dorp and others 2020). However, one particular mutation or rather SNP variant, G614, that is located outside the RBD, has become the dominant SARS-CoV-2 strain in the pandemic, while the D614 strain was initially dominant in East Asia (Korber and others 2020). In vitro, the G614 mutation increases viral load, and it is likely but not yet entirely clear whether it is clinically more infectious than D614 (Grubaugh and others 2020); it does not increase disease severity which correlates with older age (Korber and others 2020). The lack of correlation with disease severity/older age is similar but not exactly what one would expect if G614 caused increased prevalence of anosmia, since COVID-19-related anosmia is associated with younger age (von Bartheld and others 2020). Taken together, at present it is unclear whether mutations or genetic variability within or near the RBD of the virus’ spike glycoprotein can increase the likelihood of infection of the olfactory epithelium and thereby influence susceptibility to olfactory deficits; however, this question requires further attention (Forster and others 2020; Grubaugh and others 2020; Zhang and others 2020), and we expect that future studies will soon determine whether the G614 mutation may contribute to the differences in anosmia prevalence.

The second factor that may contribute to different susceptibility among populations is genetic variation of the host proteins which allow virus binding and entry. Anosmia in COVID-19 is known to have a considerable heritable component (48%, Williams and others 2020), possibly due to polymorphism and alternative splice variants of the ACE2 entry protein. Indeed, there is evidence for genetic differences in ACE2 between Asians (especially East Asians) versus Europeans (Benetti and others

2020; Cao and others 2020; Strafella and others 2020). So far, these studies have focused on expression of ACE2 variants in lung tissue and compared them with respiratory disease severity, and there are no studies yet that have compared expression of ACE2 variants in the olfactory epithelium with the prevalence of anosmia. In addition to ACE2, the TMPRSS2 protease, which facilitates virus entry, also contains variants which differ in frequency between populations, with Europeans having much higher levels of pulmonary expression than populations from East Asia (Dos Santos and others 2020). The polymorphism rs2285666 in ACE2 may be of particular interest, because its minor allele frequency is relatively high, it has a distinct geographical distribution, and it was predicted to affect ACE2 expression levels (Asselta and others 2020). The virus binding affinity, viral load in the nose, virus spreading, and thus infectivity may differ between populations, based on the frequency of variants in both ACE2 and TMPRSS2 proteins.

The possibility of splice variants of ACE2 is consistent with recent data showing that electrophoretic mobility of ACE2 expressed in (murine) olfactory epithelium differs from ACE2 expressed in brain, suggesting some tissue-specific differences in posttranslational modifications or cell-type specific ACE2 variant expression (Bilinska and others 2020). Although requiring further investigation, this suggests that subtle differences exist between ACE2 expressed in the olfactory epithelium and ACE2 expressed in other respiratory epithelial cells. Possible genetic differences in ACE2 variants or post-translational modifications such as glycosylation may contribute to varying susceptibility to anosmia caused by SARS-CoV-2 (Li and others 2005; Li and others 2020a). Such variations should be considered among the reasons why some COVID-19 patients experience anosmia as the only sign, without any significant respiratory symptoms, because in these patients the ACE2 variant present in the olfactory epithelium may bind the virus with higher affinity than the ACE2 that is present in the epithelial cells of the lower respiratory tract.

Populations with a higher binding affinity of the ACE2 receptor or with more abundant TMPRSS2 for virus entry will have higher viral loads in the nasal epithelium and therefore are more likely to become super-spreaders, making it more difficult to control the pandemic, than populations that have ACE2 receptors with lower virus binding capability and lower viral loads in the nasal epithelium. Similar to the effects of blood type on the risks of COVID-19 disease severity (Ellinghaus and others 2020), genetically determined variants of ACE2 and/or TMPRSS2 may, in part, explain the more rapid spread of the virus in Western countries, as compared with East Asia. Thus, genetic differences of the host receptors may contribute to the varying success in managing the

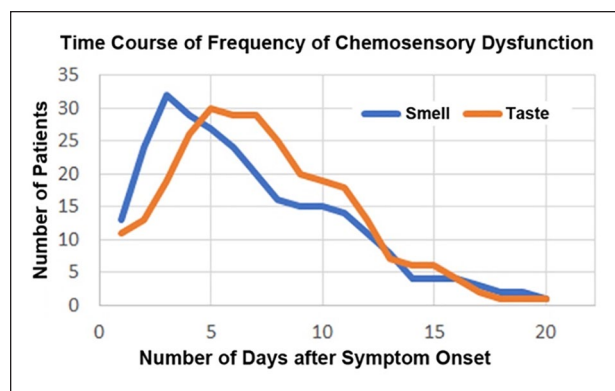


Figure 2. The time course and frequency of chemosensory dysfunctions in COVID-19 patients according to a study from Italy (Vaira and others 2020a). Note that dysfunction of smell peaked slightly earlier than dysfunction of taste, and most deficits resolved within 8 to 10 days after the peak.

COVID-19 pandemic, besides the well-known cultural, social and political differences in strategies of containment and attitudes about social distancing and use of protective measures such as face masks.

Anosmia in COVID-19: What Is the Underlying Mechanism?

The anosmia induced by SARS-CoV-2 has several unique features. Its high prevalence (in Western countries) is remarkable, as is its sudden onset, its rather short duration, and in most cases, a rapid recovery, as well as the fact that the anosmia (and taste dysfunction) can be the only symptoms in a significant fraction of patients, and that the anosmia often presents without nasal congestion or rhinorrhea. The chemosensory deficits are typically transient and last from several days to about 2 weeks (most resolve or significantly improve within 7–10 days, Lechien and others 2020a; Lee and others 2020; Printza and Constantinidis 2020; von Bartheld and others 2020). One study reported that smell is lost slightly earlier (peak on day 3) than taste (peak on days 5–7, as illustrated in Fig. 2; Vaira and others 2020a). The timing and duration of the sensory deficits are so unique in COVID-19, they may give important clues about the potential underlying mechanism, as discussed below.

The rather distinct clinical features of the anosmia differ dramatically from the SARS pandemic in which only one single case of anosmia was reported (Hwang 2006), versus literally millions of cases in COVID-19 (von Bartheld and others 2020). This is surprising, because the two viruses have considerable genomic identity of 79% to 82% (Wu and others 2020a). So what is it at the cellular and molecular level in COVID-19 that enables such a potent effect on the senses of smell and taste?

Four different principal scenarios have been considered to explain the smell dysfunction in COVID-19 patients, as illustrated in Figure 3A-E: (1) nasal obstruction/congestion and rhinorrhea, (2) loss of olfactory receptor neurons, (3) brain infiltration affecting olfactory centers, and (4) damage of support cells in the olfactory epithelium. We will evaluate for each scenario to what extent the proposed mechanism is consistent with, or supported by, the available data.

1. Many viral infections cause nasal obstruction, congestion and rhinorrhea, thereby impeding odorant access to the sensory epithelium and preventing the binding of the odorants to olfactory receptors (Doty and Mishra 2001; Hummel and others 2017). This possibility of physical obstruction (conductive olfactory loss) was initially considered a likely explanation of the anosmia in COVID-19 (Eliezer and others 2020; Gane and others 2020; Qiu and others 2020), but has now been all but ruled out by several studies, primarily because a large fraction (nearly 60%, von Bartheld and others 2020) of patients with anosmia do not have nasal congestion, obstruction or rhinorrhea (Kaye and others 2020; Lechien and others 2020b; Printza and Constantinidis 2020; Tong and others 2020; Vaira and others 2020b; von Bartheld and others 2020; Xydakis and others 2020), and because these patients lack any significant mucosal swelling of the nasal cleft or sinuses on radiographic imaging (Naeni and others 2020).
2. Does the virus infect olfactory receptor neurons, leading to their death? Such a sensorineural olfactory loss has been considered a plausible explanation of the anosmia (Baig and others 2020; Meinhardt and others 2020; Sia and others 2020; Ueha and others 2020; Wang and others 2020b). However, at closer look, there are three major inconsistencies with this scenario: the time course of cellular regeneration versus clinical recovery, the lack of expression of viral entry proteins, and the absence of the virus within olfactory neurons. When olfactory receptor neurons die, their replacement requires 8 to 10 days (Brann and Firestein 2014; Schwob 2002; Schwob and others 1995), plus about 5 days for cilia maturation (Liang 2020), but the time course of smell recovery in COVID-19 often is less than one week (Dell’Era and others 2020; Kaye and others 2020; Lee and others 2020; Printza and Constantinidis 2020; Sayin and Yazici 2020; Sedaghat and others 2020; Vaira and others 2020a; von Bartheld and others 2020). Thus, functional recovery after anosmia often is faster than the time it takes for

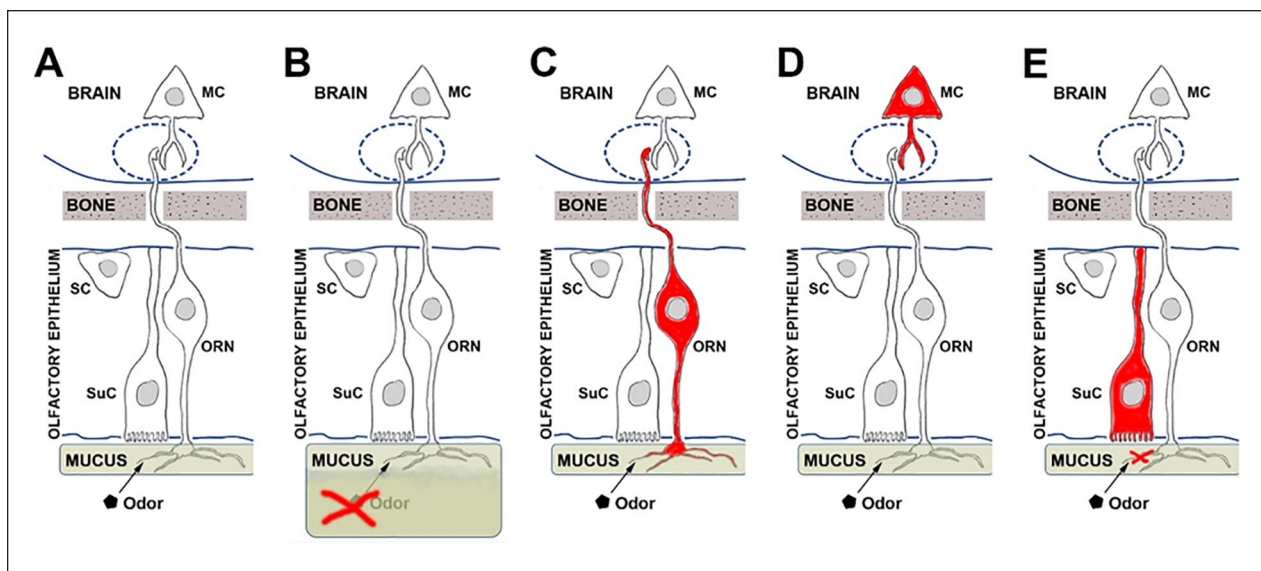


Figure 3. Schematic of the olfactory pathway to the brain with four different scenarios how the SARS-CoV-2 virus may cause anosmia or hyposmia. (A) Normal pathway: odorant molecules bind to the olfactory receptor neuron (ORN), the ORN transmits the smell sensation through the cribriform plate (bone) to the mitral cell (MC) in the olfactory bulb of the brain. Olfactory epithelium also contains support cells (sustentacular cells, SuC) and stem cells (SC) that can regenerate SuCs and ORNs. (B) Odors may not reach the ORNs, because of nasal obstruction/congestion by increased mucus. (C) The transmission of odor sensation may be blocked because of damage and/or death of ORNs. (D) The sensation of smell may be compromised because the virus affects neurons in the brain. (E) The transmission of odor sensation may be compromised, because the SuC (which assists the ORN with odor processing) is damaged by the virus.

neuron replacement, cilia maturation, and the growth of the new axons from the olfactory epithelium through the cribriform plate to form synapses in the olfactory bulb (Bryche and others 2020; Liang 2020; Schwob 2002; Soler and others 2020; Fig. 4). Regarding expression of the virus entry proteins, Butowt and Bilinska (2020) were the first who predicted, based on in-silico data, that mature olfactory receptor neurons do not express ACE2, and therefore are not likely to be infected by SARS-CoV-2. They were also the first to localize the virus entry proteins to distinct cell types in the olfactory epithelium (Bilinska et al. 2020; Table 2). There is now an emerging consensus that mature olfactory neurons do not express the virus entry proteins, ACE2 and TMPRSS2, at least not at significant levels, and not in the large majority of the mature olfactory neurons in mouse and human (Baxter and others 2020; Bilinska and others 2020; Brann and others 2020; Chen and others 2020b; Fodouljian and others 2020; Gupta and others 2020; Klingenstein and others 2020; Ziegler and others 2020—see Table 2). A recent study that localized the SARS-CoV-2 virus in the hamster olfactory epithelium confirmed this notion by showing that sustentacular cells contained the virus, but not olfactory neurons (Bryche

and others 2020). This means that the olfactory neurons are not the initial and primary target of the virus. Taken together, these facts seem to rule out that many cases of anosmia in COVID-19 can be explained by direct virus-induced damage and death of olfactory receptor neurons, although death of the olfactory neurons is likely involved in prolonged cases of anosmia.

- Does the virus infiltrate the brain, possibly from the nose, and affect olfactory centers (olfactory bulb and cortex), thereby reducing smell sensations? This scenario has been considered by several investigators (Aragão and others 2020; Baig and others 2020; Briguglio and others 2020; DosSantos and others 2020; Gilani and others 2020; Karimi-Galougahi and others 2020; Lechien and others 2020a; Li and others 2020b; Meinhardt and others 2020; Politi and others 2020; Sia and others 2020). The sudden loss of smell (and taste), followed by a rapid recovery, is a strong argument against this possibility, as is the fact that the olfactory neurons, which constitute one direct route to the brain by anterograde axonal transport, do not express the obligatory entry proteins for the virus (as detailed above). No study to date has shown that the olfactory receptor neurons or olfactory bulb

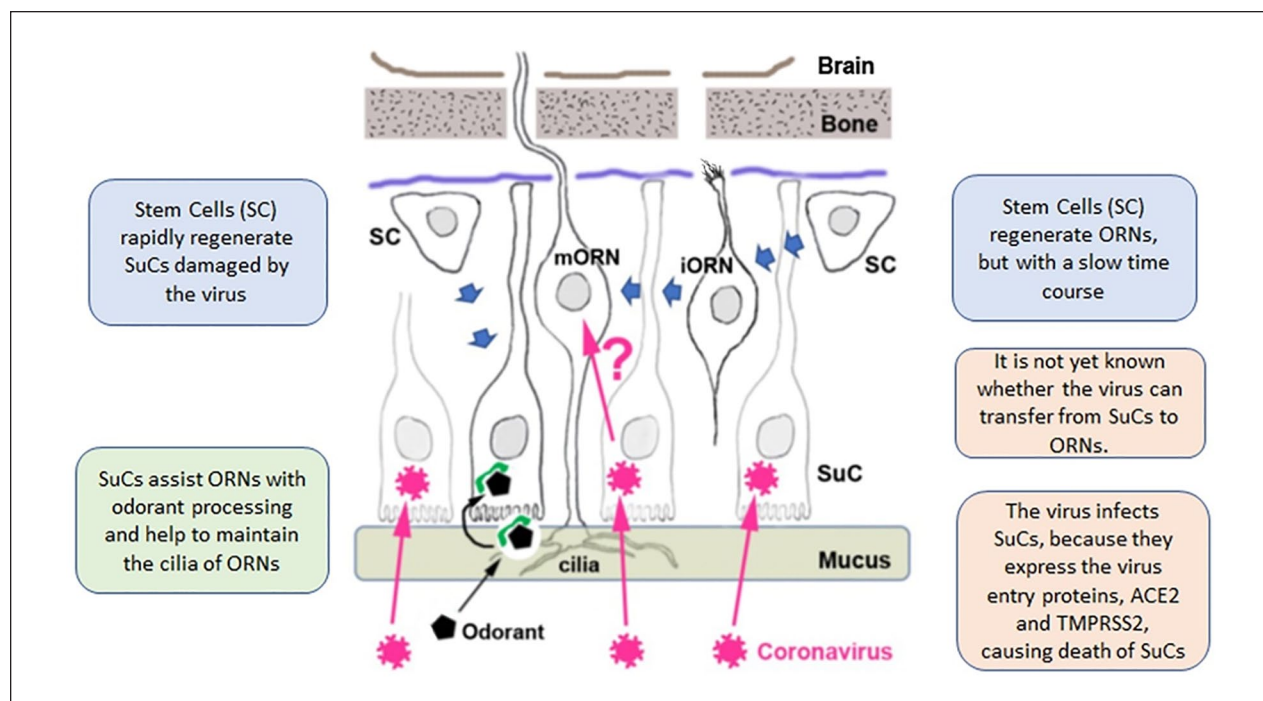


Figure 4. Entry of the SARS-CoV-2 virus in the olfactory epithelium and the virus' predicted effects that may explain the anosmia in COVID-19 patients. Coronavirus enters (pink arrows) and accumulates in the sustentacular cells (SuC) which abundantly express ACE2 and TMPRSS2 proteins, the entry proteins of the virus. SuCs normally partake in the processing of the odorants by endocytosing the odorant-binding protein complex (green-black symbol), by detoxifying, by maintaining the cilia of mature olfactory receptor neurons (mORN), and by maintaining epithelial integrity. Olfactory sensation is impaired when these essential SuC functions are disrupted. It is unknown whether the virus may transfer from SuC to mature olfactory receptor neurons (mORN) which lack ACE2 and TMPRSS2 proteins (Table 2), but have axons extending to the brain. Both the SuC and mORN can be replaced by stem cells (SC—blue arrows), although SuC replacement is much faster than replacement of mORN where SC first generates immature ORN (iORN) whose axons have to grow through the bone to the brain.

neurons accumulate the virus acutely in normal (non-genetically modified) animals, at least not within the first 2 weeks after infection (Bryche and others 2020). Accordingly, the third scenario is highly unlikely to explain the often rapid and transient anosmia in COVID-19. There is currently no evidence that the SARS-CoV-2 virus itself can reach the brain through the olfactory route in the acute phase of anosmia; alterations of brain tissues by magnetic resonance imaging were not a consistent finding and may have been caused by virus-induced inflammation or by vascular/systemic routes (Aragão and others 2020; Cooper and others 2020; Politi and others 2020; Sedaghat and others 2020; Wang and others 2020b). The data from genetically modified mouse models, as discussed below, are inconsistent regarding brain infiltration (Bao and others 2020; Sun and others 2020a; Sun and others 2020b).

4. Could the virus produce damage to the support cells in the olfactory epithelium and thereby

diminish rapidly, but transiently, the sense of smell? This mechanism is supported by the abundant expression of the two entry proteins, ACE2 and TMPRSS2, in sustentacular cells in the olfactory epithelium (Figs. 4 and 5; Bilinska and others 2020; Brann and others 2020; Chen and others 2020b; Klingenstein and others 2020—Table 2), and by the presence of the virus primarily, if not exclusively, in the sustentacular cells (Bryche and others 2020; Meinhardt and others 2020). The initial reports of ACE2 expression in sustentacular cells based on RNAseq reported that only between 1% and 3% of these cells expressed ACE2 (Brann and others 2020; Ziegler and others 2020), while immunocytochemistry indicated that the large majority, if not all sustentacular cells contain ACE2 protein (Table 2). The most likely explanation for this discrepancy is that RNAseq is an inadequate technique for quantification and estimation of the extent of protein expression (Brann and others 2020). Interestingly, death of sustentacular cells does not seem

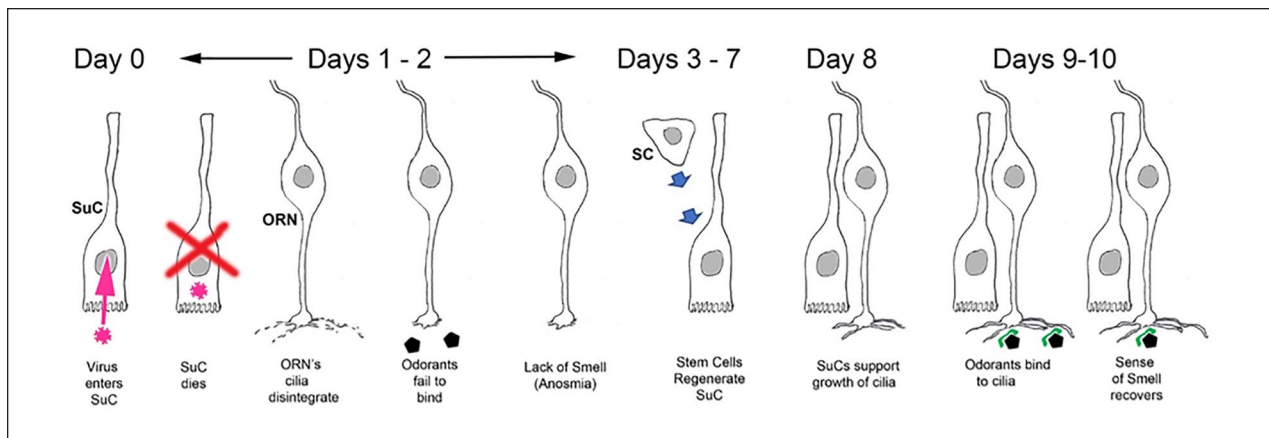


Figure 5. Time course of cellular events that may cause loss of smell and its recovery in COVID-19 patients. Day 0 = day of infection. Symbols and abbreviations are the same as explained in Figures 3 and 4. SuC, sustentacular cell; ORN, olfactory receptor neuron; SC, stem cell.

Table 2. Chronology and Specifics of the Evidence for Expression of SARS-CoV-2 Entry Proteins ACE2 and TMPRSS2 in Identified Cell Types of the Olfactory Epithelium.

Reference	Date of Publication	Species	Technique	ORN	SuC	ORN	SuC
				ACE2	ACE2	TMPRSS2	TMPRSS2
<i>Peer reviewed</i>							
Bilinska and others 2020	May 7, 2020	Mouse	ISH			+/-	+ (most)
		Mouse	ICC	-	++ (most)		
Ziegler and others 2020	May 28, 2020	Mouse	RNAseq	-	+/- (<1%)	-	+
Brann and others 2020	July 28, 2020	Mouse	RNAseq	-	+/- (<3%)	-	+ (~50%)
		Mouse	ICC	-	++ (most)		
		Human	ICC	-	+		
<i>Preprints—not yet peer reviewed</i>							
Gupta and others 2020	April 1, 2020	Human	RNAseq	-	+/- (<1%)		
Fodouliau and others 2020	April 2, 2020	Human	RNAseq	-	+	-	+
Chen and others 2020b	May 9, 2020	Human	ICC	-	++ (most)		
Baxter and others 2020	May 15, 2020	Mouse	RNAseq	-		+/-	
Klingenstein and others 2020	July 15, 2020	Human	ICC	-	++ (most)	-	++ (most)

ORN = olfactory receptor neuron; SuC = sustentacular cell; RNAseq = RNA sequencing of single cells; ISH = in situ hybridization; ICC = immunocytochemistry; - = lack of expression; +/- = very low expression or small subpopulation; + = some expression; ++ = heavy expression

to necessarily cause death of olfactory receptor neurons; the study by Bryche and others (2020) has shown that the neurons' cilia (the dendritic extensions of the olfactory receptor neurons that bind the odorant molecules) can transiently retract or lose protein expression, implying temporary neuronal dysfunction despite persistence of olfactory nerve axons. Death and regeneration of sustentacular cells occurs much faster than death and regeneration of olfactory neurons (Bryche and others 2020; Jia and others 2010; Schwob 2002), which have to mature their dendrites and grow new axons through the cribriform

plate into the olfactory bulb (Fig. 4). Therefore, rapid replenishment of sustentacular cells is consistent with the rapid recovery of the sense of smell that is clinically observed in most cases (Fig. 2). Is damage or inactivation of sustentacular cells in the olfactory epithelium sufficient to cause functional deficits of smell sensation and is it consistent with the time course and the peculiarities of the impairment reported by COVID-19 patients? The answer to these questions requires some background knowledge about the multitude of functions that sustentacular cells may perform in the olfactory epithelium.

Does the Coronavirus Cause Anosmia by Selectively Damaging Support Cells in the Olfactory Epithelium?

Sustentacular cells have been proposed to be involved in peripheral processing of odorants in multiple ways. They appear to endocytose (clear) the odorant-binding proteins after signal transduction at the neurons' cilia to allow the next round of odorant receptor binding, thereby increasing sensitivity (Heydel and others 2013; Strotmann and Breer 2011). Sustentacular cells express multiple CYP450-family monooxygenases, which hydroxylate and help to remove toxic volatiles (Heydel and others 2013). Sustentacular cells may supply neuronal cilia with some of the glucose required to meet the high energy demands of the olfactory transduction cascade (Cooper and others 2020; Villar and others 2017). Sustentacular cells also maintain the structural integrity of the olfactory epithelium (Bryche and others 2020; Jia and others 2010). Hence, these support cells are closely associated, both metabolically and functionally, with olfactory neurons and with odorant signal transduction (Fig. 4).

Recently, the SARS-CoV-2 virus was localized after nasal infection, and the time course of its effects on the olfactory system was determined (Bryche and others 2020). The virus localized exclusively to sustentacular cells and caused a massive degeneration of the olfactory epithelium and a widespread loss of the sustentacular cells, along with the olfactory cilia. The rapid loss of the sustentacular cells was reminiscent to that seen when the olfactory epithelium was treated with nickel sulfate in neurotoxic concentrations—most of the olfactory axons remained intact, implying that many olfactory receptor neurons survived (Bryche and others 2020; Jia and others 2010). The cilia began to recover within 7 to 10 days after infection (Bryche and others 2020). This suggests that the odorants would fail to bind to their cognate odorant receptors until cilia are structurally and functionally restored (Liang 2020). Sustentacular cells appear to be essential for the maintenance and normal function of the cilia extending from the knobs (Fig. 4).

Accordingly, the coronavirus-induced anosmia or hyposmia may be a direct effect of the virus on the function of sustentacular cells, by reducing the odorant clearing function, or they may be indirect, by causing secondary metabolic or other dysfunction of the olfactory receptor neurons, since the sustentacular cells also serve to protect these neurons. Sustentacular cells regenerate after damage with a faster rate than olfactory receptor neurons (Schwob 2002; Schwob and others 1995; Fig. 4), which may explain why the COVID-19 anosmia is usually short lasting (Fig. 2).

It is tempting to speculate that a similar function of support cells exists in the taste buds, since the taste defects occur with a very similar time course as the olfactory defects (Lee and others 2020; Vaira and others 2020a; Fig. 2), but there are no studies yet available to support this hypothesis, and a recent study using RNAseq did not find significant ACE2 or TMPRSS2 expression in mouse taste buds (Wang and others 2020e). As an alternative or supplement to the virus-induced destruction of sustentacular cells, there may be consequences of immune cell infiltration from the basal lamina into the olfactory epithelium. Immune cell infiltration by macrophages and lymphocytes has been shown for mammalian and human olfactory epithelium infected by SARS-CoV-2 (Bryche and others 2020; Meinhardt and others 2020), and this appears to be accompanied by a significant increase in the levels of the proinflammatory cytokine, tumor necrosis factor alpha (Torabi and others 2020). It has been suggested that inflammation-mediated loss of odorant receptor expression may contribute to the anosmia in COVID-19 (Rodriguez and others 2020; Torabi and others 2020; Yan and others 2020). The potential roles of inflammation in olfactory dysfunction was recently reviewed (Oliviero and others 2020; Rodriguez and others 2020).

It is curious that three recent studies reported contradictory findings about virus accumulation. Two studies claimed that the virus was present in some olfactory receptor neurons, in human and hamster (Meinhardt and others 2020; Sia and others 2020), while another study reported that, in hamster, the virus was present exclusively in sustentacular cells (Bryche and others 2020). How can this be reconciled? The former studies did not identify cell types in the olfactory epithelium, they only visualized the virus, and their interpretation of virus being located in olfactory neurons is questionable: in the Meinhardt study, the authors apparently mis-identified obliquely sectioned sustentacular cells for olfactory neuron processes in their figure 4A ("knobs" are much too large), as also noted by Cooper and others (2020). Accordingly, the data presented by Meinhardt and others (2020) and Sia and others (2020) may be consistent with those of Bryche and others (2020), who employed double labeling with cell-type specific markers to unambiguously identify the virus-containing cell types in the olfactory epithelium.

Why do some COVID-19 patients have longer-lasting anosmia? While the large majority regain their sense of smell within 1 to 3 weeks, there are reports of some patients remaining anosmic or hyposmic for months or more. The most likely explanation is that in those cases, a larger area of the sensory epithelium was affected, possibly with a more profound destruction of the epithelium that included death of a larger number of olfactory

receptor neurons. The extent of epithelial destruction varied in both the human and animal studies (Bryche and others 2020; Meinhardt and others 2020).

Taken together, it is most likely that the anosmia and hyposmia observed in COVID-19 patients is caused by viral entry, infection, and death of sustentacular cells, which does not necessarily lead to infection, damage, death, and the need for regeneration of olfactory receptor neurons. Therefore, the scenario (4), specific elimination of the function of sustentacular cells, is the most likely mechanism for the transient smell dysfunction in COVID-19. What would be needed for definitive proof of this hypothesis? Histological examination (biopsies) of human olfactory epithelium during progressive stages of COVID-19 infection, ideally by comparing biopsies from cases with anosmia and biopsies from cases without anosmia.

Implications for Early Diagnosis, Viral Loads, and Virus Spreading

Several studies have reported that the nasal epithelium, and in particular the olfactory epithelium, expresses large amounts of the novel coronavirus entry proteins, ACE2 and TMPRSS2 (Bilinska and others 2020; Brann and others 2020, Table 2). The abundance and the localization of the expression of the entry proteins may be responsible for the higher viral loads in nasal epithelium than in oral mucosa or throat respiratory epithelium (Hou and others 2020; Meinhardt and others 2020; Rockx and others 2020; Wang and others 2020c; Zou and others 2020), and this may explain why dysfunctions of smell and taste are rapid, immediate, and often the only symptoms in otherwise asymptomatic carriers of COVID-19. These studies further indicate that the sustentacular cells are the first to be infected by SARS-CoV-2, and apparently are responsible for the large viral load (Bryche and others 2020; Hou and others 2020; Meinhardt and others 2020; Rockx and others 2020). The abundant expression of entry proteins in the olfactory epithelium, together with the predicted high viral load in this tissue, has implications for the preferred location to obtain swabs for viral testing: Taking swabs from the pharynx may yield less virus than taking swabs from the nasal epithelium, thereby increasing sensitivity of the test and decreasing the number of false negatives (Butowt and Bilinska 2020). The extraordinarily high viral load in the nasal epithelium also explains why many of the otherwise asymptomatic COVID-19 carriers may be the super-spreaders responsible for much of the COVID-19 transmission (Oran and Topol 2020). Loss of smell is a symptom, which is particularly relevant for the infected young and working population who is likely to spread the disease faster. Rapid identification of these individuals, for example, by

using smell monitoring mobile apps, could be very relevant to reducing pandemic spread (Menni and others 2020a).

Anosmia as a Diagnostic Tool

Since many otherwise asymptomatic carriers of COVID-19 have reductions in smell and/or taste, and since such an impairment is one of the earliest symptoms, it has been suggested that olfactory/gustatory deficits could be used as a valuable screening tool and for a preliminary diagnosis (Bénézit and others 2020; Hopkins and others 2020; Parma and others 2020; Sedaghat and others 2020; Tong and others 2020; Tudrej and others 2020; Yan and others 2020). Such a screening is relatively cheap, and very fast, and could be implemented together with a subsequent gene- or protein-based test for viral particles. This approach may be more sensitive than temperature checks, given the relatively large percentage of COVID-19 patients in Western countries who do not present with a fever (Grant and others 2020). Quantitative analysis of more than 76,000 users of COVID-19 Symptom Study app revealed that the predictive ability of loss of smell and taste to be higher than fever or persistent cough (Menni and others 2020a). Certainly, olfactory deficits will not be entirely specific for COVID-19, as they may be associated with other viral and nonviral insults, but when rapid screening is needed, it may prove useful to distinguish between potentially infected and non-infected individuals. Above predictions were already tested by olfactory researchers. Population screening by Menni and others (2020b) based on developed smartphone app suggested that loss of sense of smell and taste could be included as part of routine screening for COVID-19 and should be added to the symptom list currently developed by the World Health Organization. Additionally, few other online platforms such as SmellTracker (developed in Noam Sobel's laboratory) are currently used for self-monitoring of smell for detecting early signs of COVID-19.

There are several reports which indicate that a support-cell induced olfactory impairment may differ from an impairment caused by simple nasal congestion, or by damage to the olfactory receptor neurons. This may be because the sustentacular cells are likely involved in termination of the odor binding (clearance of odorant-binding proteins, Heydel and others 2013, as discussed above), and therefore may predominantly alter the threshold of sensation (intensity of odors, Dell'Era and others 2020; Rodriguez and others 2020; Vaira and others 2020a; Walsh-Messinger and others 2020). Another aspect that needs to be considered is that the expression of ACE2 is not uniform throughout the nasal epithelium, but it shows a gradient with greater expression in the

dorsal region, and less in the ventral region (Brann and others 2020). Since there is some topography in the location of different classes of odorant receptors in the olfactory epithelium (Sakano 2010; Vedin and others 2004), and some aspects of odorant perception such as hedonics are topography dependent (Kermen and others 2016), it can be speculated that the SARS-CoV-2 induced destruction of sustentacular cells may affect some aspects of odor processing and perception (e.g., intensity, aversiveness, attractiveness) more than others. In fact, altered odor hedonics was recently demonstrated in asymptomatic students in pandemic hot spot (Walsh-Messinger and others 2020). This could possibly lead to a characteristic profile of hyposmia that may be detectable by careful testing. If this atypical profile can be clinically differentiated from the “garden variety” anosmia or hyposmia, then this approach may provide useful diagnostic information. Although the current state of knowledge about the combinatorial odor coding in mammalian olfactory system has no bearing on this hypothesis, it is worth exploring this direction because we still do not know all the key mechanisms achieving odor detection at the molecular level.

Is There a Route for SARS-CoV-2 from the Nose to the Brain?

The SARS-CoV-2 virus has been shown to be present in the brain parenchyma and cerebrospinal fluid in humans (Meinhardt and others 2020; Moriguchi and others 2020; Paniz-Mondolfi and others 2020; Wu and others 2020b) and in some of the animal models (Jiang and others 2020; Sia and others 2020; Sun and others 2020b, Table 3), but it is still unclear how the virus manages to get there. The possible routes include three main pathways: Neuronal, by moving along cranial nerves (nervus terminalis, olfactory, trigeminal, facial, glossopharyngeal, vagal); vascular/systemic, mediated via endothelial cells or leukocytes that cross the blood-brain barrier; and gaining access to cerebrospinal fluid-containing spaces; or a combination of some of these three pathways (Briguglio and others 2020; Dubé and others 2018; Li and others 2020b; Plakhov and others 1995; Zou and others 2020; Zubair and others 2020). We will focus in this section on the potential routes through the cribriform plate. Many investigators have discussed the possibility that SARS-CoV viruses infect the brain through an olfactory route (Baig and others 2020; Butowt and Bilinska 2020; Gilani and others 2020; Li and others 2020b; McCray and others 2007; Meinhardt and others 2020; Netland and others 2008; Sia and others 2020; Ueha and others 2020; Zhou and others 2020; Zubair and others 2020). Less often, it is remembered that a second cranial nerve enters the brain through the

cribriform plate: the nervus terminalis. Intuitively, the olfactory and terminal nerves are a plausible pathway to the brain, because these neurons are the only cranial nerve neurons that have a peripheral dendritic process with direct access to the virus in the nasal cavity, and a central axon that reaches the brain, without any synaptic transfer through a pseudounipolar ganglion cell as in the other sensory systems. The four possible routes from the nose to the brain, through the cribriform plate, are illustrated in Figure 6A-D.

The Olfactory Nerve

It is now well established that most of the olfactory receptor neurons do not express the virus entry proteins, ACE2 and TMPRSS2 (Baxter and others 2020; Bilinska and others 2020; Brann and others 2020; Chen and others 2020b; Fodoulian and others 2020; Gupta and others 2020; Klingenstein and others 2020; Table 2), and consistent with the absence of the entry proteins, there is no convincing evidence that olfactory receptor neurons accumulate SARS-CoV-2, neither in animal models (Bryche and others 2020; Sia and others 2020) nor in humans (Meinhardt and others 2020). But just because the olfactory receptor neurons do not express the two entry proteins for the virus, or only at very low levels (TMPRSS2), is it safe to assume that SARS-CoV viruses cannot utilize the olfactory route to the brain? Unfortunately, the answer is no. There is circumstantial evidence that SARS viruses can move beyond sustentacular cells and can reach the brain. One can consider several potential mechanisms that may allow such a transfer to happen. We know that the virus can and does readily enter sustentacular cells (Bryche and others 2020). A key question is: how can the virus possibly transfer from sustentacular cells to either the olfactory neurons or to other cells or structures that allow it to gain access to the cerebrospinal fluid? Is close vicinity between cell types sufficient to transfer SARS-CoV-2 to a neighboring cell that is not synaptically connected (Fig. 4)? If this indeed happens, the virus may utilize an organelle exchange system (exosome pathway) between support cells and neurons, as has been shown to exist between donor and host cells in other systems (Sadeghipour and Mathias 2017). Another mechanism has been proposed by DosSantos and others (2020), using the information that some stem cells in the olfactory epithelium express low levels of ACE2 (Krolewski and others 2013; Brann and others 2020; Durante and others 2020; Fodoulian and others 2020). It is—at least theoretically—possible that the virus may move from sustentacular cells to stem cells, which generate immature olfactory receptor neurons, and when these turn into mature olfactory receptor neurons (with axons extending

Table 3. Genetically Modified Mouse Models Expressing Human ACE2.

Short Name (Box 1)	Level of Expression	Promoter	Site of Integration	Technology	SARS-CoV in Brain	Reference
“Tseng mouse”	High overexpression	Artificial CAG	Random	Expression cassette Microinjection	Not evaluated	Tseng and others 2007
“Perlman mouse”	High overexpression	Cytokeratin K18	Random	Expression cassette Microinjection	Yes	McCray and others 2007; Netland and others 2008
“Qin mouse”	Mild overexpression	Exogenous murine ACE2	Random	Expression cassette Microinjection	No	Yang and others 2007; Bao and others 2020
“Baric mouse”	High overexpression	FOXJ1	Random	Expression cassette Microinjection	Yes (but only in deceased mice)	Menachery and others 2016; Jiang and others 2020
“Sun mouse”	Physiological	Endogenous murine ACE2	Knock-in (ACE2 locus)	CRISPR/Cas9 elements microinjection	Yes	Sun and others 2020b
“Zhao mouse”	Transient overexpression	Viral CMV	No integration to host genome	Recombinant adenoviral vector transduction	No	Sun and others 2020a

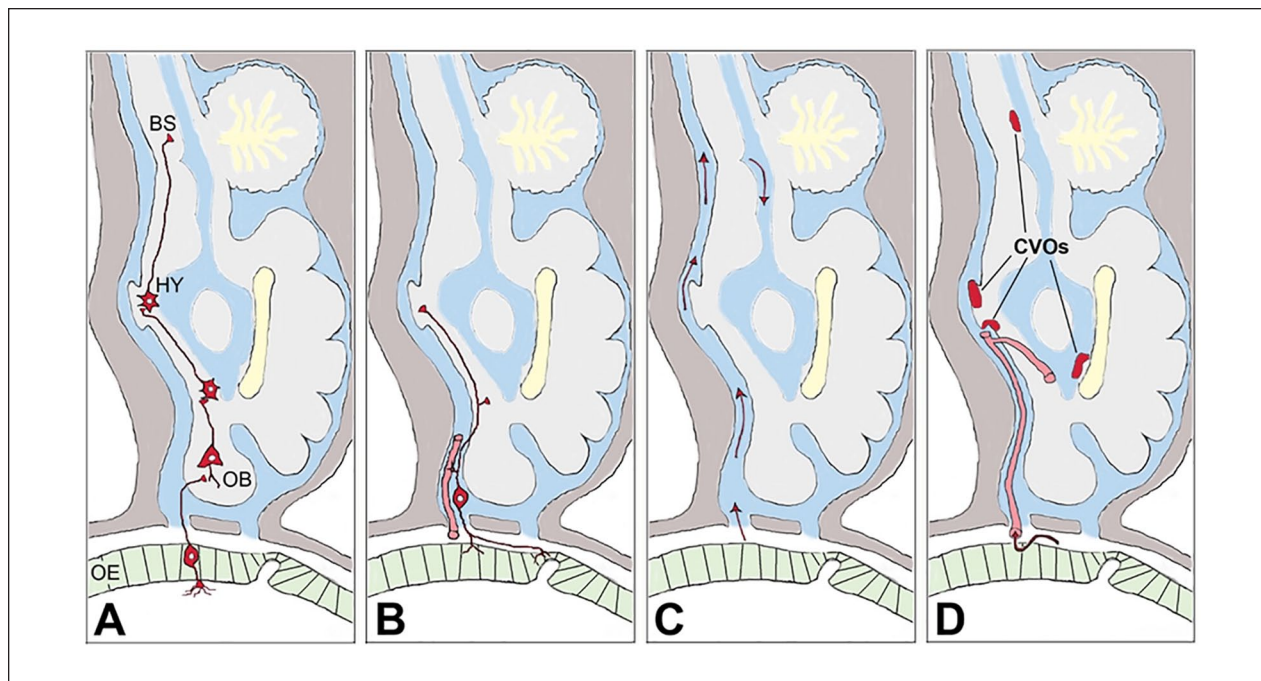


Figure 6. Four potential routes of SARS-CoV-2 virus from the nose to the brain through the cribriform plate. (A) Olfactory circuits. (B) Nervus terminalis. (C) Cerebrospinal fluid. (D) Vasculature. BS, brainstem; CVOs, circumventricular organs; HY, hypothalamus; OB, olfactory bulb; OE, olfactory epithelium.

into the olfactory bulb), they may transfer the virus directly to the olfactory bulb and beyond (Fig. 6A).

The Nervus Terminalis (Cranial Nerve “0”)

All mammals have a collection of neurons with cell bodies dispersed along the course of the olfactory nerve and olfactory bulb that are thought to have chemosensory and/or autonomic/endocrine functions, including regulation of mucous secretion in the nasal mucosa. They connect the nasal epithelium with brain centers caudal to the olfactory bulb—the medial forebrain (septum), preoptic area, and hypothalamus (Larsell 1950). These neurons are relatively sparse in humans (30–1500 cells); they are much larger in number in some marine mammals (10,000–20,000, Larsell 1950; Oelschläger and others 1987). Whether these cells express ACE2 or TMPRSS2 is currently not known, but ACE2 expression may be hypothesized, based on the presumed function of regulating blood flow in marine mammals (Oelschläger and others 1987). In mouse, the nervus terminalis cells are known to innervate not only blood vessels (including fenestrated capillaries)—thus resembling circumventricular organs—but some of the cells are also in direct contact with the subarachnoid space (Jennes 1987). These properties make the nervus terminalis a nearly ideal conduit for SARS-CoV-2 transmission to caudal brain centers, to the cerebrospinal fluid, and into the vascular system (Fig. 6B), especially if virus entry proteins are expressed.

The targets of the nervus terminalis—including the hypothalamus—may also transfer the virus in the brain parenchyma via ACE2-expressing neurons (Nampoothiri and others 2020; Pal and Banerjee 2020). SARS-CoV-1 has been shown in humans and in an animal model to accumulate in the hypothalamus (Gu and others 2005; Netland and others 2008).

Cerebrospinal Fluid–Containing Spaces

Cerebrospinal fluid (CSF) drains through the cribriform plate into lymphatic vessels and this space is in immediate vicinity of, and between, the olfactory nerve fibers (Norwood and others 2019). Although the flow is primarily in the direction from the brain toward the nasal cavity, it is conceivable—and there is precedent—that some compounds can travel in the opposite direction (Lochhead and Thorne 2012). Substances that cross the nasal epithelium and reach the lamina propria may either absorb into the vasculature, or they may enter spaces between the perineural sheaths surrounding the olfactory nerve and thereby gain access to the CSF and the brain (Lochhead and Thorne 2012), as illustrated in Figure 6C.

Vascular System

The nasal passages are highly vascular. Substances that enter the blood vessels may cross the blood-brain barrier

in circumventricular organs, or they may bypass the blood-brain barrier via direct nose-to-brain pathways to enter the brain as described above. The faster transport from the nasal epithelium to the olfactory bulb and brainstem is thought to be mediated by extracellular bulk flow within perivascular spaces of cerebral blood vessels rather than via intracellular transport along cranial nerves (Lochhead and Thorne 2012). Circumventricular organs, as illustrated in Figure 6D, may take up the virus from the vasculature through ACE2-expressing tanycytes (Nampoothiri and others 2020).

Insights From Animal Models. It is known from animal models that different viruses use different pathways and combinations of pathways to invade the brain from the periphery (Dubé and others 2018; Perlman and others 1990; Plakhov and others 1995). Some viruses transfer from neuron to neuron, using anterograde and retrograde axonal transport, which takes approximately one day for a directly connected neuron (Dubé and others 2018; Netland and others 2008), while other viruses can enter spaces containing cerebrospinal fluid, for example, in openings of the cribriform plate, from where they rapidly distribute throughout the ventricular spaces in the brain and infect neurons, including some that have no direct connections with the olfactory system (Netland and others 2008; Plakhov and others 1995).

When human ACE2 was overexpressed in a mouse model using the cytokeratin K18 promoter (see Box 1), the virus responsible for SARS, SARS-CoV-1, rapidly infected the brain after intranasal inoculation (McCray and others 2007; Netland and others 2008), and the mice died within less than a week from brain infection, apparently due to death of virus-infected neurons in the brainstem. Since cytokeratin K18 happens to be expressed in sustentacular cells, but not in olfactory receptor neurons (Schwob and others 1995), the SARS virus appears to have taken a route that originated with the support cells in this mouse model, enabled by the abundant (because overexpressed) ACE2 protein in these cells. Interestingly, the pathway of SARS-CoV-1 from the nose to the brainstem could not be explained solely by olfactory neuron-to-neuron transport, because in some cases, the olfactory bulb was spared (McCray and others 2007), and because transport was too fast, and rapidly reached neurons not connected to the olfactory system (Netland and others 2008).

Taken the above into account, we expand the hypothesis proposed by Li and others (2020b): Since sustentacular cells extend the entire thickness of the olfactory epithelium, SARS-CoV-2 can gain access to the lamina propria, and may be extruded into the cerebrospinal fluid-containing spaces within the cribriform plate, and then spread rapidly throughout the ventricular system, infecting initially cell types that are close to the ventricular

ependyma (such as dorsal raphe in the brainstem, neurons in the hypothalamus and basal ganglia), but rarely reach brain parts that are remote from the ventricular system (e.g., the cerebellum). This hypothesis is consistent with the findings of both, human studies (Meinhardt and others 2020; Nampoothiri and others 2020) as well as animal models examining the neurotropism of viruses, including coronaviruses (Perlman and others 1990; Netland and others 2008).

Synthesis from Animal and Human Studies. A recent study has reported how frequently brain regions contained SARS-CoV-2 virus in COVID-19 patients (Meinhardt and others 2020). Although this study did not provide the timeline after infection, only the endpoint, it is interesting to compare with the brain nuclei that contained SARS-CoV-1 virus in the animal model (McCray and others 2007; Netland and others 2008). Of particular interest is that in humans, even though the olfactory mucosa had the highest viral load in nearly all cases that were examined (Meinhardt and others 2020), the medulla oblongata was more often positive for the virus than the olfactory bulb, which speaks against a neuron-to-neuron transfer of the virus along the olfactory nerve, and is more consistent with a spread through the cerebrospinal fluid compartment, as indicated also by the animal model (McCray and others 2007; Netland and others 2008). The study on this mouse model has also provided some information on the timing and sequence in which the virus appears in the brain after intranasal infection (Netland and others 2008). Interestingly, the arrival of the virus in the olfactory bulb did not precede other sites, as one would expect if it was transferred from olfactory receptor neurons to mitral cells and then to second- and third-order targets of the olfactory bulb, but rather appeared simultaneously in the olfactory bulb, raphe neurons in the medulla and in neurons of the hypothalamus and basal ganglia (Netland and others 2008), suggesting that the neuron-to-neuron transport was not the only route in the brain. Similarly, in humans with COVID-19 and high viral loads in the olfactory epithelium, more cases with significant amounts of detectable virus actually involved the medulla than the olfactory bulb, and they were equal between the olfactory bulb and trigeminal ganglion (Meinhardt and others 2020). Taken together with the mouse time course studies, the currently available data suggest that there probably is a transfer of the virus from the olfactory epithelium through the cribriform plate to the brain, but in addition to being anterogradely transported along axons and transferred to second-order neurons in the olfactory bulb, the virus also appears to utilize another route, likely cerebrospinal fluid spaces which penetrate the cribriform plate along with the olfactory nerve fibers, and by entering channels formed by olfactory ensheathing cells (Butowt

Box 1. Genetically Modified Mouse Models for the Study of Coronavirus Neurotropism.

Mouse models expressing human ACE2 protease, the SARS-CoV-1 and SARS-CoV-2 host receptor, are fundamental tools for progress in COVID-19 research. During the SARS epidemic several mouse models were developed. The first and best known was a mouse overexpressing human ACE2 under the epithelial-specific K18 promoter created in Stanley Perlman's laboratory (McCray and others 2007). A second mouse line, created at the Chinese Academy of Medical Sciences (Yang and others 2007), expresses human ACE2 with likely more physiological levels as its expression was controlled by a murine ACE2 promoter. Unfortunately, this second mouse model was not made commercially available. A third humanized ACE2 model was developed in Ralph Baric's laboratory (Menachery and others 2016). In this mouse line, human ACE2 is overexpressed under control of lung ciliated epithelial cell-specific FOXJ1 promoter. The "Baric mouse" was recently infected intranasally with SARS-CoV-2, and it was concluded that the symptoms observed in this model resemble human COVID-19 symptoms (Jiang and others 2020). In contrast to the "Perlman mouse", most of the infected mice recovered and did not accumulate viral particles in the brain, except in a few deceased mice. Another overexpressor mouse that uses a strong artificial CAG promoter was developed in the Tseng laboratory. However, all three hACE2 overexpressing mouse models suffer from possible artefacts caused by random transgene integration into the mouse genome, and they possibly have different pattern of hACE2 expression in the olfactory epithelium due to the usage of different promoters (Table 3). Transcriptome analysis reveals that the K18 promoter used in the "Perlman mouse" has more sustentacular cell-specific expression in the olfactory epithelium, while the FOXJ1 promoter used in the "Baric mouse" likely has more neuronal expression. However, none of the overexpressing mice developed for SARS-Cov-1 studies have spatiotemporal expression of hACE2 identical to endogenous murine ACE2. Therefore, these mice lines are not ideal models to perform clear-cut experiments elucidating SARS-CoV-2 trafficking within the olfactory pathway.

Recently, Sun and others (2020b) developed a much-anticipated humanized ACE2 knock-in mouse by using CRISPR/Cas9 technology. This mouse expresses human hACE2 under murine endogenous promoter and a transgene is inserted within the murine ACE2 locus. This in theory ensures human ACE2 expression at physiological levels and in a spatiotemporal pattern characteristic for endogenous murine ACE2. On intranasal injection of SARS-CoV-2, these hACE2 mice accumulate high viral loads not only in the lungs but also in the brain (Sun and others 2020b). In some of the mouse models, but not all, SARS-CoV was found in the brain (Table 3), but so far, the route to the brain has been investigated only in the "Perlman mouse" (Netland and others 2008). It can be expected that studies in additional mouse lines will soon be forthcoming that determine whether viral particles can transfer from the olfactory epithelium to the brain along olfactory axons or by alternative routes. Such studies will be most convincing when mouse models are used that express human ACE2 under endogenous promoters, similar to the "Sun mouse."

Another recent approach to establish a new mouse model for COVID-19 research was recently reported by Sun and others (2020a). The authors showed that reliable and transient expression of human ACE2 may be achieved by transduction with recombinant adenoviral vector. Their viral CMV promoter drives hACE2 expression mostly in pulmonary epithelial cells; thus, almost exclusively pulmonary and no neurological symptoms were observed. To use similar transient hACE2 mice in studies of SARS-CoV-2 in the nervous system, another promoter such as synapsin I must be used. The advantage of this approach is that the model may be created without time-consuming breeding.

We expect that several lines of mice will soon be created that express hACE2 within so-called "safe harbor" ROSA26 locus. Inserting the hACE2 within this locus will eliminate the possibility that the transgene causes artefacts by having effects on the expression of nearby genes (Friedrich and Soriano 1991). The use of the ROSA26 strategy will also enable mice to express hACE2 under different promoters, including native ROSA26 promoter, strong artificial promoters, and tissue-specific promoters. Tissue-specific expression of hACE2 will be possible after crossing the ROSA26-hACE2 line containing a STOP codon flanked by loxP sites with the mouse line in which Cre recombinase is controlled by a tissue-specific promoter. In addition, the creation of a recombinant SARS-CoV-2 virus containing Cre recombinase will allow tracking infected cells in vivo after infection of a reporter mouse in which the fluorescent marker protein is expressed only after removal of the stop codon by Cre. COVID-19 research requires multiple optimal mouse models. Some genetically modified lines are more suitable for vaccine and therapeutic testing, while other lines will be better suited to study SARS-CoV-2 biology in the nervous system. We predict that the aforementioned mouse models will soon contribute to significant progress in understanding the molecular mechanisms of olfactory dysfunction as well as axonal transport and brain infection in COVID-19.

and Bilinska 2020; Li and others 2020b; Norwood and others 2019; van Riel and others 2015), or by using nervous terminalis cells as conduits (Fig. 6B). Accessing the cerebrospinal fluid would allow the virus to rapidly distribute throughout the ventricular system, reaching first nuclei that have periventricular locations, such as raphe

and hypothalamus—which is where Netland and others (2008) describe heavy accumulation coincident with infection of the olfactory bulb. Animal models with physiological levels of human ACE2 expression will be valuable tools to determine the precise pathway of SARS-CoV-2 to brain infection (Box 1).

Is there ACE2-independent virus entry and transfer? For the interpretation of data obtained in the study mentioned above, it must also be considered that the novel coronavirus may utilize an ACE2-independent route to transfer from sustentacular cells to olfactory receptor neurons. SARS-CoV-1 and other coronaviruses may use additional lower-affinity co-receptors besides the main high-affinity receptor. For example, it was shown that SARS-CoV-1, in addition to ACE2, may use CD209 glycoproteins as alternative host receptors (Jeffers and others 2004). SARS-CoV-2 can utilize CD147 to enter some cell types (Wang and others 2020a). Although olfactory receptor neurons do not express any or very little ACE2, they do express CD147 as shown by multiple microarray and RNAseq studies (Krolewski and others 2013; Nickell and others 2012; Saraiva and others 2015). Therefore, it is possible that some viral particles pass from sustentacular cells to olfactory neurons by using a CD147-dependent mechanism. Alternatively, the virus may utilize an exosome pathway that is known to allow viruses to transfer between cells (Sadeghipour and Mathias 2017). Furthermore, it is possible that SARS-CoV-2 itself upregulates ACE2 in host tissues (Nampoothiri and others 2020; Ziegler and others 2020)—which adds another level of complexity in identifying relevant cell types and potential routes of infection.

Potential Consequences of Viral Brain Infection for Neurodegenerative Diseases

Once a virus has entered the brain, it can persist there for many years, and such long-term presence may lead to inflammation that is thought to play a role in chronic neurological diseases (Desforges and others 2019; Dubé and others 2018). These are additional reasons why it is important to better understand whether and how the novel SARS-CoV-2 virus may utilize a route through the cribriform plate to the brain. COVID-19 patients can present with a variety of neurological symptoms (Mao and others 2020; Wang and others 2020b). The long-term presence of the virus in the brain may lead to inflammation and perhaps initiate or aggravate chronic neurological diseases such as multiple sclerosis and Parkinson's disease (Desforges and others 2019; Dubé and others 2018; Serrano-Castro and others 2020). The pathway from the nose to the brain must be considered among other potential routes of SARS-CoV-2 from the periphery to the brain (Baig and others 2020; Butowt and Bilinska 2020; DosSantos and others 2020; Li and others 2020b). Given that the olfactory epithelium has such intense expression of the entry proteins for the SARS-CoV-2 virus—the highest expression level in the nasal cavity—and is located on the

main route of infection through aerial spread, neurologists need to be vigilant to the possibility of brain infection by SARS-CoV-2 using a route from the nose to the brain.

Conclusion

In summary, the olfactory/gustatory dysfunctions of COVID-19 patients provide both, daunting challenges due to the early, very high viral load and possibilities of super-spreading and a nasal route to brain infection, and also potentially fortunate opportunities, namely to utilize anosmia as a rapid screening tool to identify early, and otherwise asymptomatic, carriers of the novel coronavirus. An emerging field of interest and a major novel hypothesis is that genetic differences in the prevalence of chemosensory defects may be caused by variations in the binding affinity of the ACE2 receptor for the virus and therefore may dictate infectivity and spreading of the virus. Differences between populations in this regard remain to be verified by future studies, but if confirmed, they would have considerable implications for defining which populations are most vulnerable to COVID-19 infection and how to best and most effectively manage the pandemic by a customized approach, that takes into account the infectivity of different populations. Whether a nasal route to the brain exists for SARS-CoV-2, especially after prolonged virus exposure, requires further studies, and it will be important to precisely define the short-term and potential long-term consequences of SARS-CoV-2 in the brain.

Acknowledgments

The authors thank Matthias Bochtler (International Institute of Molecular and Cell Biology, Warsaw) for helpful comments.

Author Contributions

Both authors contributed equally to the writing of this article.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This work was supported by the “Excellence Initiative—Research University” ID-UB program at the Nicolaus Copernicus University and by the National Institutes of Health. CSvB is supported by a grant from the National Institute of General Medical Sciences (GM103554). The funders had no role in the preparation, review, or approval of the manuscript or decision to submit the manuscript for publication.

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