The host response to severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection can result in prolonged pathologies collectively referred to as post-acute sequelae of COVID-19 (PASC) or long COVID. To better understand the mechanism underlying long COVID biology, we compared the short- and long-term systemic responses in the golden hamster after either SARS-CoV-2 or influenza A virus (IAV) infection. Results demonstrated that SARS-CoV-2 exceeded IAV in its capacity to cause permanent injury to the lung and kidney and uniquely affected the olfactory bulb (OB) and olfactory epithelium (OE). Despite a lack of detectable infectious virus, the OB and OE demonstrated myeloid and T cell activation, proinflammatory cytokine production, and an interferon response that correlated with behavioral changes extending a month after viral clearance. These sustained transcriptional changes could also be corroborated from tissue isolated from individuals who recovered from COVID-19. These data highlight a molecular mechanism for persistent COVID-19 symptomology and provide a small animal model to explore future therapeutics.

INTRODUCTION
Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a respiratory RNA virus that emerged in 2019 and is associated with a variety of clinical phenotypes ranging from asymptomatic to more severe disease generally referred to as coronavirus disease (COVID-19) (1). In most cases among young and healthy individuals, COVID-19 is characterized by a relatively mild flu-like illness and includes limited respiratory tract congestion, fever, myalgia, headache, and anosmia (2–4). Among older populations, especially males and those with comorbidities, COVID-19 can result in severe respiratory distress, multiorgan complications, and death (1, 5).

Regardless of age or underlying health, virus infection is thought to impair host transcriptional and translational processes to enhance replication (6–8). As a result, infected cells are unable to elicit a type I interferon (IFN-I) response, a central mediator of the host’s antiviral defenses through the up-regulation of hundreds of antiviral IFN-stimulated genes (ISGs) (9, 10). During a SARS-CoV-2 infection, induction of IFN-I largely derives from uninfected cells, such as resident macrophages and other phagocytic cells (11). Despite blocking many aspects of the host antiviral response, SARS-CoV-2 infection relies on persistent signaling of the nuclear factor kB (NFkB) transcription factor family, indirectly culminating in transcriptional activation of proinflammatory cytokines and chemokines such as interleukin-6 (IL-6) and CXCL10, respectively (9, 12, 13). As a result of these dynamics, neutrophils and monocytes amass in the respiratory tract as the virus propagates in an environment with suboptimal antiviral defense engagement, further exacerbating the inflammatory environment. Virus infection results in extensive damage to the bronchial epithelium and pulmonary edema, ultimately leading to a loss of normal lung function (2–4).

Characterization of SARS-CoV-2 biology has identified angiotensin-converting enzyme 2 (ACE2) and a subset of proteases that enable viral entry (14–16). Despite the expression of these host factors on multiple tissues, productive SARS-CoV-2 infection appears to be largely contained in the respiratory tract (2–4). Selective localization in the airways, however, is not a product of viral tropism; rather, it is a by-product of the systemic IFN-I response that initiates at the site of infection, enabling distal tissues to become recalcitrant to subsequent infection. For example, human organoid models have demonstrated productive infection of diverse tissues ex vivo despite being rarely observed in vivo (17–19). This phenomenon can also be modeled in the golden hamster, one of the most widely used small animal models for COVID-19, which demonstrates consistent infection of the respiratory tract and olfactory epithelium (OE) upon intranasal challenge, with only sporadic isolation of virus from other tissues unless IFN-I biology is disrupted (20–24). This finding is further supported by the fact that SARS-CoV-2 has been seen to readily infect ex vivo organotypic cultures of hamster brain tissues, which are not readily seen to be sites of viral replication in intranasal infection.

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conditions (21, 25). This same phenomenon can be observed when infected individuals are immunosuppressed (17, 26–28). Although it remains unclear how common infection of distal tissues occurs during a SARS-CoV-2 infection, system-wide inflammation is consistent (2–4). Together, these data suggest that the molecular underpinnings of acute COVID-19 are a by-product of the damage caused by the virus and the systemic response that ensues.

In most individuals, virus infection is successfully cleared with the appearance of neutralizing antibodies to the spike attachment protein. In general, the appearance of the humoral response correlates to resolution of the symptoms associated with SARS-CoV-2 (29–31). However, a growing body of evidence suggests that, in a subset of individuals, SARS-CoV-2 infection results in prolonged complications including shortness of breath, persistent fevers, fatigue, depression, anxiety, and a state of chronic impairment of memory and concentration known colloquially as “brain fog.” The direct cause of these impairments, known collectively as “long COVID” or post-acute sequelae of COVID-19 (PASC), is currently unknown (32, 33).

To better understand the prolonged effects caused by SARS-CoV-2 infection, we focused on the golden hamster as a model system. The hamster model has proven to largely phenocopy COVID-19 biology without any requirement for SARS-CoV-2 adaptation and has demonstrated a propensity to display severe lung morphology and a tropism that matches what is observed in human patients (20, 21, 23, 34). In these studies, we characterize the host response to SARS-CoV-2 and benchmark the findings to a former influenza A virus (IAV) pandemic virus infection (35).

Here, we show that although both IAV and SARS-CoV-2 induce a systemic antiviral response, only the latter infection results in a sustained inflammatory pathology that extends well beyond clearance of the primary infection. Because this sustained inflammation also correlates with behavioral abnormalities, we propose that this biology may underlie the cause of long COVID in both humans and hamsters.

RESULTS
SARS-CoV-2– and IAV-infected hamsters develop a host response that mirrors human biology and resolves within 2 weeks after infection

To define the unique characteristics of SARS-CoV-2 infection that may contribute to persistent symptomatology, we performed a longitudinal study in hamsters infected with either SARS-CoV-2 [USA-WA1/2020; 10⁵ plaque-forming units (PFU)] or IAV (pandemic H1N1 isolate A/California/04/2009; 10³ PFU). Inoculation dosages were informed by past studies to achieve comparable kinetics and viral load in these two model systems (21, 36). Longitudinal study data demonstrated that both respiratory RNA viruses could replicate in the lungs of the golden hamster, albeit showing a modest difference with regard to clearance, consistent with what has been reported elsewhere (Fig. 1, A and B, and fig. S1, A and B) (21, 37). The delayed clearance of SARS-CoV-2 further coincided with a diminished appetite, because this cohort of hamsters gained weight more slowly than either the phosphate-buffered saline (PBS)–treated or IAV–infected animals, as we have reported elsewhere (36). IAV challenge resulted in peak titers of 10⁷ PFU/g of lung tissue on day 3 followed by a sharp decline in infectious material, showing a complete loss of infectivity by 7 days postinfection (dpi) (Fig. 1A). For SARS-CoV-2, we also observed peak viral titers at 3 dpi (about 10⁸ PFU/g), which persisted until day 5 before declining (Fig. 1B). Despite the difference in sustained virus replication upon reaching peak virus titers in both model systems, no infectious virus could be isolated on day 7; however, RNA remained detectable through quantitative reverse transcription polymerase chain reaction (qRT-PCR) for the nucleoprotein of influenza (NP) as well as the subgenomic RNA of nucleocapsid (sgN) from SARS-CoV-2 (Fig. 1, A and B, and fig. S1, A and B). On the basis of these data, we focused on day 3 to compare the acute host response to these two respiratory infections, because this time point appeared to be the most comparable between the two viruses with regard to kinetics and viral load during the disease course. Furthermore, as 3 dpi corresponded to peak infection for both viruses, we hypothesized that this time point would likely be the point in which pathologies attributable to direct viral activity would be most visible.

To compare the pathology resulting from IAV versus SARS-CoV-2, we examined cross sections of the hamster lung at 3 dpi using various histological techniques that were evaluated by a board-certified pathologist (Fig. 1, C to F, and fig. S1C). Hematoxylin and eosin (H&E) treatment of lung tissue derived from hamsters infected with either IAV or SARS-CoV-2 revealed large areas of intense staining (Fig. 1C, black stars, and fig. S1C) that, at higher magnification, implicated hypercellularity and the infiltration of inflammatory cells into both alveolar compartments and bronchial airway spaces (white stars). To better characterize the cellular content of the pulmonary inflammatory infiltrate, immunohistochemical (IHC) staining was used to label macrophages, which express ionized calcium binding adaptor molecule 1 (IBA1) (Fig. 1D), neutrophils, which express myeloperoxidase (MPO) (Fig. 1E), and T cells, which express CD3 (Fig. 1F) on these same cross sections. These efforts demonstrated that the high hematoxylin-stained regions of the lung, regardless of virus, showed extensive positivity for all three cell types, with neutrophils and macrophages predominating (Fig. 1, C to F). One notable difference between these virus models was that SARS-CoV-2 induced pulmonary infiltration that was centrally located around bronchioles and larger airway structures (Fig. 1C, black star).

In contrast to the lung, examination of distal tissues, including kidney and heart, showed moderate to no pathological features at 3 dpi for either virus (fig. S1, D and E). We observed no signs of cellular infiltration in the kidney at this time point, whereas in the heart, an organ often associated with COVID-19 complications (38), we noted some evidence for inflammation and leukocytic infiltrate in response to both viruses (fig. S1E, green stars). Together, these data suggest that the hamster phenocopies many of the histological characteristics seen in the human response to IAV or SARS-CoV-2 during acute infection (4).

To characterize the molecular dynamics of these model systems, we next performed RNA sequencing (RNA-seq) on infected lungs isolated at 3 dpi (Fig. 1G and fig. S1, F and G). These data identified about 100 differentially expressed genes (DEGs) with a P-adjusted (P_adj) value of less than 0.1 in both SARS-CoV-2– and IAV–infected lungs. Gene set enrichment analyses (GSEA) against hallmark gene ontology sets implicated activation of the IFN-I, IFN-II, tumor necrosis factor–α (TNF-α), and IL-2 signaling pathways in response to either infection (fig. S1H). These data could be further corroborated by IHC of fixed lung tissue probed for the ISG MX1 (Fig. 1H). We next examined the transcriptional response to SARS-CoV-2– and IAV–infected hamsters at 14 dpi, about 1 week after clearance (Fig. 1, A, B, and G). In contrast to the inflammation observed at 3 dpi, sequencing SARS-CoV-2– or IAV–infected lung tissue at 14 dpi showed...
Fig. 1. SARS-CoV-2 and IAV infections induce clinically representative lung pathology and are cleared by 14 dpi in the hamster model of disease. (A and B) Titer data were computed as plaque-forming units per gram (PFU/g) of lung from hamsters infected with (A) IAV (A/California/04/2009) (n = 4 per time point) or (B) SARS-CoV-2 (USA/WA1/2020) (n = 4 per time point) on days indicated. Day 0 is representative of uninfected hamsters (n = 3). (C) H&E staining of hamster lungs exposed to PBS (mock), IAV, or SARS-CoV-2 at 3 dpi. Histological analysis at various magnifications denotes hypercellularity (black stars) and infiltrate presence in bronchioles and alveoli (white stars). Scale bar size is denoted above the images. (D to F) Immunohistochemical labeling for (D) IBA1, (E) MPO, and (F) CD3 was used to label macrophage, neutrophil, and T cell populations, respectively, in the lungs of mock-, IAV-, and SARS-CoV-2–infected hamsters at 3 dpi. The size of the inset scale bars matches length described in column headers. (G) RNA-seq of lungs from SARS-CoV-2– and IAV-infected hamsters was evaluated at 3 and 14 dpi. Heatmap depicting log2 fold change of type I interferon (IFN-I) response genes (derived from HALLMARK_INTERFERON_ALPHA_RESPONSE gene set) compared to mock-infected animals was generated for these groups. (H) Immunohistochemical labeling for the IFN-stimulated gene MX1 was assessed in lungs of mock-infected, IAV-infected, or SARS-CoV-2–infected hamsters at 3 dpi. Scale bars, 250 μm.
minimal signs of an antiviral response (Fig. 1G). Together, these data demonstrate that the golden hamster model shows a robust acute response in the respiratory tract that successfully resolves both IAV and SARS-CoV-2 infection.

Transcriptional profiling of peripheral organs reveals differences between active or resolved IAV and SARS-CoV-2 infections

To corroborate the clinical validity of the SARS-CoV-2 acute hamster data, we compared our RNA-seq analyses to published results from lungs of COVID-19 deceased individuals that still had high viral loads at the time of death (Fig. 2, A to C) (39). In agreement with the published data, we found that transcriptional signatures from both groups were dominated by a marked up-regulation of the IFN-I and TNF-α signaling pathways (9, 13, 21). Because the host response to acute IAV and SARS-CoV-2 was comparable in the respiratory tract, we next sought to characterize distal tissues and expand our characterization to time points representing both active and resolved infections. Although COVID-19 symptoms usually resolve within 4 weeks after infection onset, symptoms can persist much longer in a subset of patients. Patients demonstrating symptoms lasting longer than 4 weeks after infection have now been clinically defined as having long COVID or PASC (40). To this end, we conducted additional transcriptional profiling on the lung (blue), heart (black), and kidneys (red) from hamsters infected with SARS-CoV-2 or IAV at 3 and 31 dpi, the latter being a time point where any symptom-generating pathology would be clinically defined as long COVID in a human patient (Fig. 2, A and B, and fig. S2, A to C). These data were also cross-referenced to matching tissues derived from human cadaver specimens that were infected with COVID-19 at the time of death, as described elsewhere (Fig. 2C) (41). These comparisons encompassed more than 50 samples at both early and late time points, which clustered based on tissues from which they derived (fig. S2D).

To first assess the acute response in a more systemic fashion, we used GSEA to characterize curated ontology gene sets from the aforementioned tissues. These efforts implicated a strong acute induction of the IFN-I response [false discovery rate (FDR) q < 0.0001] in all three organs after either SARS-CoV-2 or IAV infection that were also evident in corresponding human tissues (Fig. 2, A to C, and fig. S2, E to G). In addition, IFN-I signatures in the lungs of hamsters and COVID-19 cadavers were also accompanied by up-regulation of IFN-1–associated pathways, including NFκB- and IL-6–associated target genes (fig. S2, H to J). Other enriched pathways induced by SARS-CoV-2 or IAV infection included positive regulation of complement activation in the kidney and negative regulation of calcium channel formation in the heart, although these enrichments were relatively minor in comparison to the IFN-I signatures (fig. S2, E to H). Together, these data corroborate independent published studies and provide further support for the use of the golden hamster as a model for acute SARS-CoV-2 pathology (20, 21).

Having established the hamster as a clinical proxy for systemic acute pathology in response to a respiratory infection, we next sought to extrapolate these findings to any possible long-term consequences. To this end, we performed similar analysis on lung, heart, and kidney tissues collected from hamsters at 31 dpi, representing a time point greater than 2 weeks after disease resolution of the lungs (Figs. 1, A and B, and 2, D to F). In agreement with the clearance of virus, these analyses failed to show any enrichment of IFN-I– or chemokine-related signatures in any of the tissues examined (Fig. 2, D to F). Instead, tissue-specific annotations identified various biological processes involved in kidney resorption capacities and heart metabolism (Fig. 2, D and E). In the lung, GSEA at 31 dpi implicated general pathways of repair and regeneration (Fig. 2F). Among these was the biogenesis of cilia and airway repair in the lung after infection, which drives the up-regulation of genes involved in axoneme assembly and filament sliding, which are also involved in pathways that were identified as “sperm motility” (Fig. 2F).

To further visualize the development of the respiratory ontologies over time, we combined all the sequencing performed on 3, 14, and 31 dpi and mapped their significance values (Fig. 2, G to L). These data supported our earlier findings that SARS-CoV-2 and IAV infections were resolved by day 14, because the IFN-I response and neutrophil chemotaxis induced by these viruses showed a lack of enrichment at both days 14 and 31 after infection, despite their strong induction at 3 dpi (Fig. 2, G and H).

Next, we assessed microtubular motor activity and ciliary assembly ontologies over this longitudinal comparison to better understand the dynamics of bronchiolar repair, given that this biology was enriched in both viral infection models at 31 dpi (Fig. 2F). A longitudinal query for ciliary-related ontologies showed no enrichment of these same transcripts during the acute phase of the two infection models, but a disparity between the cohorts 1 week after clearance (14 dpi; Fig. 2, I and J). At this time point, SARS-CoV-2–infected lungs uniquely displayed a negative enrichment of microtubular motor activity and a negative enrichment of axoneme assembly ontologies. Because ciliary loss is part of the acute lung pathology after respiratory virus infection (21), we found the decline of microtubules and axoneme assembly–related genes specifically in response to SARS-CoV-2 noteworthy. These findings suggest that SARS-CoV-2–induced transcriptional aberrations may still be prevalent past 14 dpi, even in the absence of infectious virus or proinflammatory transcriptional profiles. These data suggest that SARS-CoV-2–induced damage may be more severe and persist for a longer duration in the respiratory tract as compared to IAV. However, by 31 dpi, the increase in microtubular motor activity and axoneme assembly likely reflect active regeneration of the ciliary machinery. This biology also corresponds with the down-regulation of genes associated with extracellular matrix assembly and collagen trimer–related genes, which are involved in tissue regeneration (Fig. 2, K and L) (42–44). The shared trends observed for the GSEAs suggest a resolving repair response at 31 dpi after either IAV or SARS-CoV-2 infection.

Histological characterization of lung, heart, and kidney tissue reveals differences in response to SARS-CoV-2 and IAV infection at 31 dpi

To assess long-term organ damage independent of the transcriptional response, we profiled lung, heart, and kidney by histological analyses after IAV or SARS-CoV-2 infection at 31 dpi (Fig. 3 and fig. S3). H&E staining revealed that both SARS-CoV-2– and IAV–infected lungs maintained their general structure but displayed numerous abnormalities. Most prominent among these pathologies was peri-bronchiolar metaplasia (also known as lambertosis) (black stars), a clinical finding in which alveolar epithelial cells undergo metaplastic transformation to become bronchiolar epithelium—like in appearance (Fig. 3A). This process generally occurs in response to severe respiratory trauma and can result in functional respiratory defects (45–47). In SARS-CoV-2–infected lungs, peribronchiolar metaplasia
Fig. 2. Transcriptional profiling of peripheral organs reveals differences between active or resolved IAV and SARS-CoV-2 infections. (A to C) Lungs (blue), kidneys (red), and hearts (black) of SARS-CoV-2-, IAV-, and mock-treated hamsters were harvested at 3 dpi and transcriptionally profiled by RNA-seq. Differential expression analysis was conducted between infected and mock-infected groups with DESeq2 and analyzed by gene set enrichment analysis (GSEA) for enrichment of indicated gene sets. Enrichment analysis results for all three tissue types are displayed in a GSEA enrichment plot for (A) IAV versus mock and (B) SARS-CoV-2 versus mock comparisons. (C) Similar transcriptomic analyses were conducted on RNA-seq data generated from human lung, heart, and kidney samples obtained from the postmortem tissues of COVID-19–infected and control donors. Results from enrichment analyses are shown as a GSEA enrichment plot. (D to F) Differential expression analysis of RNA-seq data derived from (D) lungs, (E) kidneys, and (F) hearts of SARS-CoV-2–, IAV–, and mock-infected hamsters at 31 dpi. Differential expression results were assessed using GSEA to test for enrichment of gene sets present in the MSigDB C5 gene set collection, which contains curated gene sets derived from the gene ontology resource. Significant ontological enrichments for SARS-CoV-2 versus mock differential expression analysis were further processed by REVIGO to remove redundant enrichments. The highest ranked nonredundant positive and negative enrichments for each organ are plotted by their normalized enrichment score (NES) (line magnitude) and significance [−log10(FDR q value)] (dot size). GSEA enrichment for these same gene sets in IAV versus mock differential expression data for the same tissue is plotted side by side for comparison.

GSEA from lung sequencing data from IAV–, SARS-CoV-2–, and mock-infected hamster lungs at 3, 14, and 31 dpi using curated gene ontology and human phenotype ontology gene sets. Directional significance of enrichment was plotted over time for (G) IFN-I response (GOBP_RESPONSE_TO_TYPE_I_INTERFERON), (H) neutrophil chemotaxis (GOBP_NEUTROPHIL_CHEMOTAXIS), (I) microtubular motor activity (GOMF_ATP_DEPENDENT_MICROTUBULE_MOTOR_ACTIVITY), (J) axoneme assembly (GOBP_AXONEME_ASSEMBLY), (K) extracellular matrix (ECM) assembly (GOBP_EXTRACELLULAR_MATRIX_ASSEMBLY), and (L) collagen trimer–associated genes (GOCC_COLLAGEN_TRIMER). Dotted lines show the calculated statistic for FDR q = 0.05 for positive and negative enrichment; thus, any points falling outside the dotted lines have FDR q value of <0.05.
was more expansive and was visible even at minimal magnification when compared to the comparable IAV samples (white star) (Fig. 3A). Furthermore, lungs infected by both viruses showed signs of enlarged airway spaces and residual inflammation characterized by monocytes and neutrophils visible in the alveolar spaces (Fig. 3A, red stars). This residual inflammation is in agreement with our transcriptional profiling data, which found that \(Cd177\) and \(Ly6d\), neutrophil- and monocyte-associated genes, respectively, were upregulated in lungs infected with SARS-CoV-2 at 31 dpi (fig. S2A). To confirm these findings, IHC staining was performed to label macrophage (IBA1), neutrophil (MPO), or T cell (CD3) populations in histological sections of lungs of infected hamsters at 31 dpi (fig. S3, A to C). In contrast to lungs from mock-infected animals, lungs from IAV- and SARS-CoV-2–infected animals showed localized areas of
hypercellularity that stained positively for both neutrophil and macrophage populations (fig. S3, A to C). These hypercellular areas were commonly associated with areas of lambertosis, which could be distinguished by thickened alveolar walls compared to surrounding healthy and mock alveolar tissues. In addition, in line with our sequencing, which identified a moderate and resolving repair response at 31 dpi, Verhoeff Van Gieson staining, which labels collagen and elastin fibers, showed no obvious signs of fibrotic activity, collagen deposition, or elastin degradation in response to either infection (fig. S3D).

Given the persistent gene signatures on day 31 after SARS-CoV-2 infection and the histological changes observed in the lungs, we next assessed the kidney and heart by H&E staining (Fig. 3B and fig. S3D). In the heart, we observed complete resolution of leukocytic infiltrations at 31 dpi with no noteworthy histological signatures in response to infection (fig. S3E). In the kidney, however, SARS-CoV-2–infected animals displayed areas of tubular atrophy characterized by thinning of tubular cells and widening of the tubular lumen (Fig. 3B, black stars). Closer examination also revealed the presence of proteinaceous fluid in the interstitial space surrounding these tissues (red stars). Examination of the kidneys at this time point from IAV-infected hamsters showed similar pathological findings (black stars); however, the affected areas appeared smaller and less numerous than in SARS-CoV-2–infected hamsters, consistent with the notion that IAV–induced damage is less severe than that of SARS-CoV-2 in this small animal model.

To better assess the extent of infection-induced scarring, we performed quantitative morphometric analyses on these histological images. Quantification of lambertosis and airway size showed that these pathologies were greater in the lungs of SARS-CoV-2–infected animals (Fig. 3C and fig. S3F). A similar finding was also visible with respect to tubular atrophy and previous SARS-CoV-2 infection (Fig. 3D). Together, these data demonstrate that both SARS-CoV-2 and IAV infections present similar histological signatures in the lungs and in other peripheral organs. However, despite comparable host responses, we observed a greater severity of scarring in SARS-CoV-2 infection, which, given its nature, may predispose infected individuals to greater functional defects after viral clearance.

SARS-CoV-2 induces unique neural transcriptional profiles compared to IAV

Given that long COVID may also involve neurological and neuropsychiatric symptomatology (33), we next assessed the consequences of SARS-CoV-2 infection on the nervous system. For these studies, we transcriptionally profiled several areas of the nervous system from 3 and 31 dpi cohorts. More specifically, the areas surveyed included the olfactory bulbs (OBs), medial prefrontal cortex (mPFC), striatum, thalamus, cerebellum, and trigeminal ganglion (tissues collected as depicted in Fig. 4A). These areas were chosen either because of their previously documented positivity for SARS-CoV-2 transcripts in human patients (OB and trigeminal ganglion) or because of their functional importance in sensory, motor, cognitive, or affective processes—all of which have been noted to be altered in subsets of patients with long COVID (48–53). Matched tissues from hamsters infected with IAV were also collected for comparison. After tissue processing, brain regions from 3 dpi were surveyed for the presence of viral RNA. As expected, in hamsters infected with IAV, no viral RNA could be detected from the surveyed neural tissue that aligned to the IAV genome (fig. S4A). In contrast, within the SARS-CoV-2–infected hamster cohort, viral reads were readily detectable in the nervous system in a subset of animals, consistent with the findings of others (34). In one hamster, SARS-CoV-2 reads were detectable in all surveyed regions of the nervous system (Fig. 4B). Mapping of these reads to the SARS-CoV-2 genome revealed that most reads aligned to the nucleocapsid (N) transcript, potentially implicating the deposition of circulating subgenomic RNA from a peripheral infection that is dominated by N (Fig. 4B) (54). These findings are consistent with reports of human patients displaying SARS-CoV-2 RNAemia and systemic detection of SARS-CoV-2 RNA that could reflect deposition of viral-derived inflammatory material (26, 55).

To further characterize the appearance of SARS-CoV-2–genetic material in the brains of infected hamsters, a time course was conducted in which geographically distinct regions of the brain were sampled on days 1, 4, 7, and 14 after infection in both SARS-CoV-2– and mock-infected hamsters. The OB, striatum, and cerebellum were chosen for their respective positioning in the anterior, middle, and posterior sections of the cranial cavity. These regions were assessed for SARS-CoV-2 sgN transcripts by qRT-PCR and compared to the lung, the primary site of infection (fig. S4, B to E). Mirroring previous data (Fig. 1, A and B, and fig. S1, A and B), SARS-CoV-2 sgN detection in the lungs was highest on days 1 and 4. By 7 dpi, sgN detection diminished, with only negligible transcripts detectable at 14 dpi (fig. S4B). In the OB, a low abundance of sgN at 1 dpi increased through 4 dpi in two of three hamsters before dissipating over the next 7 days (fig. S4C). Moreover, striatum and cerebellum demonstrated different patterns of sgN positivity compared to both lungs and OBs. At 1 dpi, SARS-CoV-2–infected hamsters demonstrated sgN positivity in one of three tested striatum sections and in all the cerebellum samples. Beyond this early time point, however, no cerebellum or striatum sections demonstrated sgN signal that rose above background (fig. S4, D and E).

To assess whether sgN positivity was associated with triggering of an innate immune response, transcripts for Isg15, a canonical IFN-I–stimulated gene, were assessed in sampled regions by qRT-PCR (fig. S4, F to I). In general, Isg15 signal correlated with sgN positivity; in lungs, for instance, Isg15 transcripts were elevated on days 1, 4, and 7 after infection, with its peak at 4 dpi (fig. S4F). The striatum and cerebellum likewise showed induction of Isg15 signal at 1 dpi, after which expression returned to baseline, mirroring the positive sgN signal in these same tissues (fig. S4, D, E, G, and H). The OBs show similar Isg15 transcript abundance, following sgN signal on days 1, 4, and 7 after infection; however, at 14 dpi, the OB markedly shows a newly elevated Isg15 signal in the absence of any sgN positivity (fig. S4, C and I).

To better understand the functional impacts that systemic SARS-CoV-2 and IAV challenge have on the nervous system, differential expression analyses of host transcripts were subsequently conducted across all sequenced neural areas from 3 and 31 dpi after challenge with either SARS-CoV-2 or IAV and were compared to mock infection (Fig. 4, C to H). To remain consistent to the previous studies, the time points chosen included 3 dpi, representing peak infection, and a time point after viral clearance (31 dpi), wherein no infectious material could be detected from the lungs (Fig. 1, A and B). Because RNA was isolated and sequenced for whole brain regions, DEGs observed in these analyses represent a global summation of transcriptional changes across all cellular populations present within the sampled tissue. Despite this limitation, several brain areas showed...
region-specific transcriptional alterations induced by viral infection at 3 dpi. This was evident for both SARS-CoV-2 and IAV (Fig. 4, C to H). Direct comparison of SARS-CoV-2 and IAV by differential expression analyses revealed that most surveyed regions induced a very similar transcriptional profile between the two viruses at 3 dpi (fig. S4, J and K). These findings are most prominent in the striatum, where comparison of SARS-CoV-2 versus mock conditions revealed more than 3500 DEGs in contrast to the comparison of SARS-CoV-2 versus IAV, which demonstrated no notable transcriptional differences (Fig. 4D and fig. S4J). In contrast, these analyses also revealed different transcriptional profiles in many of the surveyed regions in response to SARS-CoV-2 and IAV infection at 3 dpi. These differential responses to the two viral challenges were most prominent in the thalamus, cerebellum, and trigeminal ganglion (Fig. 4, E to G, and fig. S4, J and K).

In addition, this differential expression analyses demonstrated that transcriptional programs induced in neural tissue during viral challenge persisted for at least 1 month after infection. All surveyed regions showed DEGs in response to at least one of the viruses at 31 dpi, albeit notably few changes were observed in the trigeminal ganglion (Fig. 4, C to H). These transcriptional signatures were comparable between SARS-CoV-2 and IAV in the striatum, mPFC, and...
in the thalamus and cerebellum at 3 dpi (Fig. 4I). Similarly, when synaptic signaling showed distinctive responses to SARS-CoV-2, metabolic modulation on neural tissue, ontologies relating to synaptic signaling, neuronal plasticity, and immune activation (Fig. 4I). At 3 dpi, widespread metabolic modulation encompassing changes to mitochondrial oxidative phosphorylation, protein translation, protein degradation, nucleotide synthesis, and amino acid synthesis pathways was observed within the surveyed neural tissues in response to both SARS-CoV-2 and IAV (Fig. 4I). One example from data collected from the cerebellum and the trigeminal ganglion indicated strong negative enrichment of oxidative phosphorylation, indicating a reduced production of transcripts necessary for the assembly of the electron transport chain and mitochondrial function. Conversely, the striatum and the thalamus demonstrated inverse trends in response to both SARS-CoV-2 and IAV infections, showing an increase in oxidative phosphorylation among other metabolic ontologies. These trends were also found to be dynamic as demonstrated by an inverse relationship at 31 dpi, with cerebellum and trigeminal ganglion showing an increase in oxidative phosphorylation in contrast to striatum and mPFC, where these signatures become negatively enriched (Fig. 4I). These data suggest a dynamic process of metabolic changes that occur throughout the central nervous system (CNS) in response to viral challenge.

To better understand the underlying biology responsible for the observed transcriptional changes taking place during infection, we again performed an unbiased GSEA. These efforts implicated four general host response signatures, including metabolism, synaptic signaling, neuronal plasticity, and immune activation (Fig. 4I). To further confirm these findings, we performed immunostaining for MX1 on sections taken from the OBs of hamsters, either mock-treated or infected with SARS-CoV-2 or IAV at 3 and 31 dpi (Fig. S5A). These data supported our transcriptome findings and demonstrated elevated MX1 at both 3 and 31 dpi in response to SARS-CoV-2, with immunolabeling remaining in the periphery of the OBs (fig. S5A). In addition to ISGs, SARS-CoV-2 infection was also found to induce prolonged chemokine induction, as shown by the elevated expression of Cxcl10 and Ccl5, among others (Fig. 5, E and F, and fig. S5B).

We next sought to determine the composition of immune cells participating in the prolonged inflammatory response. To this end, we analyzed transcriptomic data from OBs at 31 dpi to identify enriched gene sets that enable deconvolution to identify the specific cell types present. These analyses showed profound enrichment for microglial and myeloid lineage gene sets, specifically in the SARS-CoV-2–infected hamsters at this time point (Fig. 5G). This analysis was further supported by a directed GSEA, which identified an enrichment in markers for microglial activation (fig. S5C). To better assess how CNS–specific cell types were changing in response to SARS-CoV-2 infection at this time point, gene sets were created for neuronal and glial cell populations using previously described cell type markers (56). These efforts further demonstrated enrichment of microglial–specific transcriptomic signatures in OBs from SARS-CoV-2–infected hamsters at 31 dpi (fig. S5D). In contrast to immune cells, gene sets identifying neuronal populations demonstrated negative enrichment at this same time point, in agreement with studies focused on the molecular basis of anosmia (57).

To further corroborate the transcriptional signatures of the OBs in response to either SARS-CoV-2 or IAV, we performed independent qRT-PCR validation and IHC on genes for which commercial antibodies for the hamster were available. These efforts illustrated a recruitment of microglial and macrophage populations to the OBs, as measured by Aif-1 transcripts, uniquely in response to SARS-CoV-2 (Fig. 5H). We next aimed to assess myeloid cell activation using histology. Immunolabeling for IBA1, the protein encoded by Aif-1, demonstrated increased myeloid positivity around the periphery of the OB, implicating a role for both microglia and possibly infiltrating macrophages, consistent with what has been reported previously during acute infection (57). This finding was most pronounced in OBs from SARS-CoV-2–infected hamsters at 3 dpi but could still be seen at 31 dpi (fig. S5, E and F). Microglia and infiltrating macrophages expressing IBA1 could be visualized clustering around the periphery of the OBs in both SARS-CoV-2– and IAV–infected hamsters, showing darker staining for IBA1 and more enlarged and rounded cell bodies (fig. S5F). On the basis of transcriptional signatures, SARS-CoV-2 infection resulted in greater activation of IBA1–stained cells, further supporting the enhanced inflammatory state as compared to IAV (fig. S5C) (58).

In addition to implicating microglial and myeloid lineages in the inflammatory phenotype observed in response to SARS-CoV-2, a positive enrichment of T cell signatures was also noted from these samples. To assess the degree of T cell infiltration involved in the hamster response, OBs from 31 dpi were immunolabeled for CD3 (fig. S5G). Staining was noticeably sparse, with minimal numbers of cells (about 20 to 50 cells per bulb) labeled positive in mock IAV, and SARS-CoV-2 OB cross sections, suggesting a lack of T cell contribution in this tissue.
To determine whether sustained IFN-I or chemokine expression was the product of a chronic infection, we next performed qRT-PCR on the OBs (Fig. 5I). These data demonstrate that, at 31 dpi, neither IAV nor SARS-CoV-2 transcripts could be detected, although SARS-CoV-2 sgN RNA was evident at 3 dpi. qRT-PCR data were further validated by RNA in situ hybridization, which confirmed that SARS-CoV-2 spike protein staining was only observed at 3 dpi in the glomerular region and was undetectable at 31 dpi (fig. S6A). To further assess whether inflammation of the OBs was associated with cellular apoptosis in this region, we performed a terminal
deoxyxynucleotidyl transferase–mediated deoxyuridine triphosphate (dUTP) nick end labeling (TUNEL) stain on mock-, SARS-CoV-2–, or IAV-infected hamsters at these times. Quantification of the total number of TUNEL-positive nuclei in the OBs revealed no differences between the infection groups at either time point, indicating that neither the acute infection nor the uniquely prolonged inflammatory patterns in SARS-CoV-2 OBs were associated with local apoptosis, in agreement with the findings of others (fig. S6B) (57).

To explore whether this proinflammatory signal was present in additional anatomical regions linked to the OB, the OE was harvested from hamsters infected with SARS-CoV-2, IAV, or PBS (mock) at 31 dpi, as this tissue has been demonstrated to harbor infectious virus (21, 37). Ontological analyses of RNA-seq data demonstrated that, similar to the OBs, the OE of SARS-CoV-2–infected hamsters uniquely showed up-regulated signatures for IFN-I and IFN-II (Fig. 5) and fig. S6C). These signatures were driven by expression of canonical ISGs such as Isg15, Mx1, Mx2, Ifit3, Ifr7, Oas2, and Bst2. However, in contrast to the OB, ontological analysis also highlighted several transcriptomic signatures implicating T cell recruitment, activation, differentiation, and immune response in the OE. Chemotactic recruitment signatures were driven by increases in expression of Ccl7, Cxcl10, Ccl5, and Ccl11 as well as other cellular migration factor genes, such as Jam1 and Rac2 (Fig. 5) and fig. S6C). T cell activation ontologies, on the other hand, were driven by the up-regulated expression of antigen presentation markers, such as Hla-dra, Wipf4, and B2m, concurrently with up-regulation of T cell–associated genes such as Jak3, Coro1A, Cd3e, Cd3g, and Cd3d (Fig. 5) and fig. S6C). In addition to immune signatures, these analyses highlighted a negative enrichment for genes relating to sensory perception of smell and olfaction capabilities, which were evident for both SARS-CoV-2– and IAV-infected hamsters (fig. S6C).

To better understand the cellular makeup of this immune response, cell type enrichment analyses were again conducted (fig. S6D). These analyses implicated the presence of unique neuroepithelial lymphocytes and macrophage populations in the OE after SARS-CoV-2 infection. Because SARS-CoV-2 has demonstrated sex-dependent biases, we also assessed whether evidence for sustained perturbations in the OBs or OE was present in female hamsters (59). To this end, a cohort of all-female hamsters was infected with SARS-CoV-2 or IAV and analyzed about 2 weeks after viral clearance (24 dpi; fig. S6, E and F). Consistent with our earlier results performed in male hamsters, we found elevated Isg15 and Ccl5 transcripts in both tissues, suggesting that the observed phenotype was not sex dependent.

**Olfactory inflammation was associated with behavioral alteration in hamsters**

Given previous findings that hamsters, similar to humans, can experience anosmia in response to SARS-CoV-2 infection (34) and the fact that injury to the OB has been linked to development of neuro-behavioral disorders such as depression (60–63), we next assessed the functional consequences of sustained neuronal perturbations, such as prolonged OB and OE inflammation in SARS-CoV-2–infected hamsters beyond 4 weeks after infection. To this end, we first looked to elucidate how SARS-CoV-2 infection affected olfaction. Hamsters infected with IAV or SARS-CoV-2 were assessed for smell and compared to a cohort of mock–infected animals. Using a food-finding test at 3, 15, and 28 dpi, we confirmed the results reported by de Melo et al. (34), showing that SARS-CoV-2–infected hamsters took longer to find buried food at 3 dpi, but showed no difference at 15 or 28 dpi when compared to mock or IAV (Fig. 6, A to C, and fig. S6, G to I). In contrast, when this same experiment was performed with readily visible food, all cohorts, at all time points tested, displayed roughly equivalent times (fig. S6, J to L). These results occurred alongside prolonged inflammation in both OB and OE and coincided with transcriptional signatures indicative of diminished expression of olfactory receptor genes for both IAV and SARS-CoV-2, in agreement with the loss of sustentacular cells during acute infection being a driver of anosmia (fig. S6C) (57).

To determine whether prolonged OB inflammation was correlated with altered metrics on assays that assess affective behaviors, mock–, IAV–, and SARS-CoV-2–treated hamsters were subjected to a marble burying assay at 26 dpi (Fig. 6D). When hamsters were subjected to this assay, an established metric for assessing rodent repetitive and anxiety-like behaviors (64), SARS-CoV-2–infected animals demonstrated a reduction in burying activity compared to the mock–treated animals, which performed comparably to the IAV–infected group (Fig. 6D). Behavioral tests such as these are believed to reflect a sign of elevated compulsiveness or anxiety-like behaviors (65). Given this evidence, this behavior suggests that SARS-CoV-2 induces behavioral changes in hamsters.

**SARS-CoV-2 infection is associated with sustained inflammatory transcriptional programs in human olfactory tissues**

Last, to ascertain whether our data could be extended to aspects of the human disease, we performed RNA-seq on postmortem OB and OE tissue (Fig. 7). These tissues derived from donors that had recovered from a medically documented history of COVID-19 infection, defined as being PCR negative for more than 1 month before death (tables S1 and S2). Tissues from healthy donors without history of COVID-19 infection were also collected as controls.

For OB tissues, two COVID–19–recovered (long post-COVID) donors and one uninfected control donor were able to be sequenced. Differential expression and gene enrichment analyses revealed the presence of proinflammatory transcriptional programs in the OBs of the recovered donors compared to control tissues (Fig. 7, A and B, and table S1). In agreement with that observed in hamsters, gene sets for complement (Fig. 7A) and IFN (Fig. 7B) were enriched. Complement gene set enrichment was driven by up-regulation of direct complement cascade genes such as C3, F8, and CIQA and complement regulator proteins S100A9, SERPINE1, and CLU (Fig. 7A). IFN ontologies were driven by shared up-regulation of ISGs such as ISG15, OAS3, ISG20, CXCL10, MX1, IFIT3, IFIT1, and IFRF as well as other immune-related genes such as those involved in antigen presentation (B2M, HLA-DQA1, and CD74) and cytokine signaling (IL7, IL6, and IL4R) (Fig. 7B). One long post-COVID donor (long post-COVID 1) showed elevated inflammatory gene expression than the other, possibly reflecting their medical history because this individual had COVID-19 within 4 months of death as opposed to the companion sample (about 6 months). For OE tissues, two additional long post-COVID donors and three uninfected control donors were able to be successfully sequenced. Differential expression and gene enrichment analyses showed that, similar to findings in OB tissues, long post-COVID donor tissue displayed enriched proinflammatory transcriptional profiles (Fig. 7, C and D, and table S2). In contrast to IFN-mediated transcriptional programs observed in the OB, the OE displayed a
higher enrichment for gene sets detailing chemotactic and T cell–specific activities. These programs were respectively driven by up-regulation of a variety of chemotactic genes (such as \textit{CCL5}, \textit{CCL8}, \textit{CCL19}, and \textit{CXCR3}) and T cell–associated genes (such as \textit{CD3D}, \textit{CD3G}, \textit{CD3E}, \textit{GATA3}, \textit{CD4}, and \textit{LY9}) (Fig. 7, C and D). Once again, one long post-COVID donor (long post-COVID 1) appeared to have a higher abundance of chemotactic and T cell markers than the other long post-COVID sample, likely reflecting the time between COVID-19 and death.

Comparison of the OBs and OE from 31 dpi derived from SARS-CoV-2–infected hamsters and long post-COVID humans demonstrated a correlation between the respective transcriptional programs. In the OB, both hamster and human SARS-CoV-2–recovered tissues show enhanced induction of IFN-II, leukocyte chemotaxis, and immune response pathways (Fig. 7E). The two organs further show coordinated metabolic programs, with both hamster and human post–SARS-CoV-2 infection tissues demonstrating up-regulation of ribosomal production (Fig. 7E). Moreover, in the OE, both human and hamster tissues demonstrate enrichment of chemotaxis and T cell functional pathways after SARS-CoV-2 clearance (Fig. 7F). The notable correlation generated when comparing transcriptional responses between hamsters and humans that have recovered from SARS-CoV-2 would suggest that the host response results in prolonged olfactory inflammation capable of affecting other areas of the brain.

**DISCUSSION**

Together, these data demonstrate that SARS-CoV-2 and IAV infections produce a wide range of longitudinal systemic impacts that include both shared and unique characteristics. In peripheral tissues such as lung, heart, and kidney, SARS-CoV-2 and IAV seem to induce similar transcriptomic and histological changes both during acute infection and after viral clearance, highlighting the importance of benchmarking the host response to SARS-CoV-2 against an independent virus challenge model before accrediting a phenotype as distinct. Despite the strength of modeling the host response to two different viruses, the trajectory of replication of SARS-CoV-2 versus IAV did restrict our comparisons to the initial peaks of both infections (day 3) and the weeks beyond virus clearance (days 14 to 31). The times between these windows involve varying rates of replication and clearance, which made direct comparisons of temporally matched samples difficult to interpret. When focusing on day 3 during the acute infection peak of both infection models, transcriptional profiles were dominated by systemically up-regulated IFN signature, indicating a robust cell-intrinsic antiviral response across nearly all tissues in response to both viruses. Despite this, histological characterization of tissues outside of the lungs during acute infection showed minimal evidence of cellular infiltration. Note that, although kidney immune infiltration and markers of viral presence have been observed in human patients, these findings have almost exclusively been documented in postmortem patients who died of
severe COVID-19 infection. Our findings here thus likely reflect a milder infection state (66). After resolution of the infection, scarring from SARS-CoV-2 infection was more severe than that observed for IAV. This severity involved a higher degree of kidney tubular atrophy and lambertosis compared to IAV. These differences likely reflect the unique biologies of each virus. SARS-CoV-2 generates substantially more double-stranded RNA (dsRNA) during its life cycle as a result of sub-genomic RNA (sgRNA) production (13, 67). Given the immunogenicity and stability of dsRNA, it seems reasonable to postulate that similar degrees of replication would result in a more robust

Fig. 7. SARS-CoV-2 infection is associated with sustained inflammatory transcriptional programs in human OB and OE.

(A and B) Radar plots derived from OB tissues collected at autopsy from healthy control donor (control) (n = 1) as well as donors that had previously recovered from clinically documented COVID-19 (long post-COVID) (n = 2). Donors were screened to only include those where COVID-19 positivity was documented greater than 1 month before autopsy. Tissues were RNA-sequenced, and long post-COVID tissues were compared to control tissues by differential expression analysis. GSEA using the Hallmark Gene sets was used to characterize transcriptomic programs. Transcripts per million read (TPM) counts for individual genes making up these responses were plotted onto radar plots. Gene expression is normalized to the highest expressing sample for each individual gene, with expression shown as the percentage of TPM value of that sample (which is shown as 100% of its own value). (A) Complement and (B) IFN responses were measured. (C and D) Analyses as described in (A) were used to characterize the transcriptional response of OE tissues harvested from long post-COVID (n = 2) and control donors (n = 3), evaluating (C) lymphocyte chemotaxis and (D) T cell selection. (E and F) GSEA enrichment plots from (E) OB and (F) OE human tissues were plotted by their NES (magnitude of line) and significance [-log_{10}(FDR q value)] (size of dot). GSEA enrichments of these same gene sets from analogous tissue analysis in hamsters (SARS-CoV-2–infected versus mock-infected OB and OE tissues at 31 dpi) were plotted beside matching human enrichment data. The numerical FDR q value of each enrichment is denoted above or below the respective NES line for that gene set.
induces the observed pathological abnormalities that would result in reduction of functional capacity in affected regions, as supported by previously reported data (45, 47, 68–71). Together with data indicating that SARS-CoV-2 induces a more prolonged inflammatory profile alongside changes in appetite, these data suggest that SARS-CoV-2 induces a more severe systemic acute infection than IAV, which our data here indicate is likely mediated by inflammatory processes shared between the two viruses (36, 37, 72).

Similar to peripheral tissues, the nervous system showed a mix of shared and unique responses to SARS-CoV-2 and IAV infection. During acute infection, both viruses induced CNS-wide IFN-I responses as well as region-specific transcriptional alterations that in some cases persisted beyond 1 month after infection. The most prominent of these alterations took place in the striatum, where both IAV and SARS-CoV-2 induced similar changes associated with metabolic and functional shifts. Preclinical and clinical literature have correlated this type of activity within striatal subregions with chronic or traumatic stress (73, 74), affective disorders (75, 76), and chronic pain states (77). These changes could partially underlie the increased clinical risk of neurological and neuropsychiatric disorder onset associated with IAV (78), SARS-CoV-2 (79, 80), and even other viruses and seemingly unrelated clinical conditions (81, 82).

In contrast to the striatum, the thalamus displays a differentially regulated response to SARS-CoV-2 and IAV infection, showing a hypoexcitable versus a hyperexcitable state, respectively. The hypoexcitable state induced by SARS-CoV-2 in the hamster model also shows many similarities to clinical reports concerning cognitive deficits and dysExecutive syndrome among patients with COVID-19 (83, 84). These data suggest that thalamic dysregulation may contribute to cognitive disruption, potentially in the form of altered intrathalamic function or functional connectivity with key brain regions that drive emotion, motivation, cognition, sleep, pain, wakefulness, and motor activity. Altered thalamic function and structure have also been previously associated with cognitive deficits in conditions such as multiple sclerosis (85), traumatic brain injury (86), and Alzheimer’s disease (87). Thalamic dysfunction may also underlie neurological conditions that have been observed in patients with long COVID including chronic pain, headache, myalgias, seizures, sleep, and affective disorders (88–94). Furthermore, transcriptional changes strongly associated with dendrite development were also seen in this region at both early and late time points after SARS-CoV-2 infection but not in response to IAV infection. Dysregulation of the key genes driving this enrichment in the SARS-CoV-2 thalamus (Nrp1, App, Crtcl, Ctnmd2, Camkk2a, Kalrn, Bmp7, Ppp1r9b, Mecp2, Cux1,Dlg4, Apm2,Ephb2, Map2k7, and Ephb1) are associated with cognitive impairments and affective disorders such as major depressive disorder when analyzed together using Enrichr’s DisGeNET function (95). Thus, regional transcriptional changes in thalamic nuclei may facilitate the development of neuropsychiatric disorders in patients recovering from SARS-CoV-2 infection.

By far, the most unique response to SARS-CoV-2 took place in the olfactory tissue. At 31 dpi, the OB of IAV-infected hamsters returned to a baseline transcriptional state, whereas SARS-CoV-2–infected hamsters appeared to be in the midst of an ongoing infection characterized by microglial and infiltrating macrophage activation and a robust IFN-I and chemokine response. This phenotype was robust, consistent, and present in both male and female hamsters. These inflammatory responses were especially unexpected given that we were unable to detect the presence of viral RNA in either the OB or lungs at this time point, and as demonstrated previously, we know that hamsters generate a strong spike protein–specific antibody response as early as 7 dpi (21, 37). IHC of MX1 corroborated our transcriptional findings and showed localization of this persistent IFN-I response to the glomerular regions of the OBs. Given that the OBs were positive for SARS-CoV-2 early in infection, these data may suggest the existence of persistent defective viral genomes or remaining debris capable of inducing a host response. Alternatively, SARS-CoV-2 infection of OBs may result in loss of a physical barrier, enabling the introduction of microbiome commensals, which could contribute to the inflammatory profile. This latter hypothesis is supported by recent evidence that SARS-CoV-2 can induce gross morphological changes in the OE characterized by thinning and sloughing of the tissue that would normally prevent bacterial commensals from interacting with the OB (96). The extent to which the olfactory system contributes to the persistent inflammatory processes described within our study remains to be determined. However, when surveying olfactory tissues from human donors that had recovered from documented COVID-19 infection but had died of other causes, we observed similar transcriptional signatures to those documented in the hamster model, suggesting a shared etiology. Despite these data, note that although we were unable to detect infectious material at 31 dpi, other reports suggest that this may be possible under some circumstances and therefore should not be ruled out as another possible contributor to long COVID symptomology (21, 34, 37).

Chronic inflammation within the OBs can affect sensory, emotional, and cognitive processes. In this study, we see that persistent inflammation in olfactory tissues of SARS-CoV-2–infected hamsters is accompanied by a change in behavior as measured by marble burying. Because the OBs are functionally connected to—and can thus influence the activity of—the limbic system, which controls appetitive, sensory, emotional, and cognitive responses, the connection between these findings suggests this to be the possible causation of changes in behavior. Previous preclinical studies link OB damage with depressive phenotypes that can be reversed with antidepressant treatment (61–63, 97). These data suggest that chronic nasal and OB inflammation may drive neurodegeneration and structural changes, consistent with long COVID symptoms (60, 98). This is further supported by recently reported clinical evidence that shows that patients that have recovered from even mild COVID-19 demonstrate loss of gray matter in limbic cortical areas functionally linked to the olfactory system and by additional clinical evidence demonstrating that long COVID presentation is associated with a variety of immune-associated risk factors (99, 100). Together, our peripheral organ and CNS findings identify transcriptional and histologic signatures caused by SARS-CoV-2 infection that may induce a variety of somatosensory, affective, and cognitive impairments that persist well past the time of original infection. Given the systemic scope of these findings, we hypothesize that they elucidate a molecular basis of much of the heterogenous symptomology that makes up long COVID.

Our findings highlight the value in the golden hamster model for its ability to phenocopy COVID-19. However, note that we do not show the direct causality between the persistent inflammation in the brain and the changed behaviors of our hamsters. Moreover, the behavioral tests conducted on our hamsters have largely been characterized in mice and rats. These studies also include fewer animals.
than generally used for behavioral testing, which is a consequence of needing to perform these studies within a high containment facility. Last, we were only able to obtain OB tissues from a few individuals for comparison to the phenotype observed in the hamster model. These comparisons were not only represented by a small sample size, but we also had to compare the modeling of a moderate infection with hamsters with severe disease outcomes in humans. Although not ideal, the ability to improve on these processes is extremely difficult because patients with moderate COVID-19 do not generally succumb to infection, making sampling of heart, kidney, or other tissues of interest not possible. Given these limitations, we cannot state that all observations documented in hamsters accurately reflect the prolonged symptomology after SARS-CoV-2 infection.

**MATERIALS AND METHODS**

**Study design**

The objective of this study was to define the systemic transcriptional response to SARS-CoV-2 and benchmark these data against IAV, both during peak viral load and after clearance. Sample size was dictated by available cage space in our high-containment facility that we maximized at every opportunity. Hamsters were always divided into three cohorts: mock treatment, IAV infection, and SARS-CoV-2 infection. In general, we used three to five animals per experimental variable being tested. Regarding data inclusion and exclusion criteria, all samples were included in the analyses unless technical issues were evident such as low-quality sequencing data. In these examples, we re-prepped the sequencing library or excluded samples as necessary. All RNA-seq experiments thus included between two and five animals per tested tissue and condition. Numbers of included animals and samples for sequencing in differential expression analyses can be found on the National Center for Biotechnology Information (NCBI) Gene Expression Omnibus (GEO) using accession number GSE203001. Any hamster experiments using numbers of samples that fall outside of this range have numbers noted in figure legends. Human cadaver samples were used only to corroborate our findings in hamsters, and we analyzed as many samples as we could obtain. All histology and behavioral experiments were performed and scored blinded. Marble burying behavioral study data were assessed for outliers, which were corrected for using iterative Grubbs’s method. The study was approved by the affiliated institutions of all authors listed. Human heart, lung, and kidney samples collected from cadavers were available through dbGAP (accession #38851 and 1D phs002258.v.1.p1). These samples were originally provided by the Weill Cornell Medicine Department of Pathology. The Tissue Procurement Facility operated under an Institutional Review Board (IRB)–approved protocol and followed guidelines set by Health Insurance Portability and Accountability Act (HIPAA). Experiments using samples from humans were conducted in accordance with local regulations and with the approval of the IRB at the Weill Cornell Medicine. The autopsy samples were considered human tissue research and were collected under IRB protocols 20-04021814 and 19-11021069. All autopsies had consent for research use from next of kin, and these studies were determined as exempt by IRB at Weill Cornell Medicine under those protocol numbers. Brain tissue and nasal epithelium, including the OE and OB, were retrieved under a collaborative effort by the Department of Neuropathology and the Department of Otolaryngology at Columbia University Irving Medical Center. The study was approved by the ethics committee and IRB of Columbia University Medical Center (IRB AAAT0689 and AAAS7370). All animal experiments were performed according to protocols approved by the Institutional Animal Care and Use Committee (IACUC; PROTO202000113 and IPROTO20210000053) and Institutional Biosafety Committee at the Icahn School of Medicine at Mount Sinai (ISMMS) and New York University Langone Health (NYULH; PROTO202100078).

**Viruses and cells**

SARS-CoV-2 isolate USA-WA1/2020 (NR-52281) (Biodefense and Emerging Infections Research Resources Repository, BEI Resources) was propagated in Vero-E6 cells in Dulbecco’s modified Eagle’s medium (DMEM) (Gibco) supplemented with 2% fetal bovine serum (FBS) (MilliporeSigma), 1 mM Heps (Lonza Bioscience), and 1% penicillin/streptomycin (Thermo Fisher Scientific). Virus stocks were filtered by centrifugation with an Amicon Ultra-15 Centrifugal filter unit (Sigma-Aldrich) and sequenced to ensure maintenance of the furin cleavage site. Infectious viral titers were quantified by plaque assay in Vero-E6 cells [American Type Culture Collection (ATCC)] in DMEM supplemented with 2% FBS, 1 mM Heps, and 0.7% Oxdoid agar (Thermo Fisher Scientific). Assays were fixed in 5% paraformaldehyde (PFA) (Thermo Fisher Scientific) and stained with crystal violet (Sigma-Aldrich). All infections were performed with either passage 3 or 4 SARS-CoV-2. IAV H1N1 isolate A/California/04/2009 (BEI Resources) was propagated in Madin-Darby canine kidney (ATCC) cells in DMEM supplemented with 0.35% bovine serum albumin (Thermo Fisher Scientific), filtered, and sequenced in a manner comparable to SARS-CoV-2 stocks. All cells used in this study were routinely tested for the presence of mycoplasma using the MycoAlert Mycoplasma Detection Kit (Lonza).

**Hamster experiments**

Six- to 7-week-old male golden Syrian hamsters (*Mesocricetus auratus*) were obtained from Charles River Laboratories. Hamsters were acclimated to the U.S. Centers for Disease Control and Prevention (CDC)/U.S. Department of Agriculture (USDA)–approved biosafety level 3 (BSL-3) facility. All animal experiments were performed according to protocols approved by the IACUC and Institutional Biosafety Committee at ISMMS and NYULH. Hamsters were euthanized by intra-peritoneal injection of pentobarbital and cardiac perfusion with 60 ml of PBS. Each tissue was harvested at 1, 3, 4, 7, 14, or 31 dpi. Collected tissues were homogenized with PBS or TRIzol (Thermo Fisher Scientific) in Lysis Matrix A homogenization tubes (MP Biomedicals) for 40 s at 6 m/s for two cycles in a FastPrep-24 5G bead grinder and lysis system (MP Biomedicals) for plaque assay or RNA isolation, respectively. Additional tissues were fixed in 4% PFA for at least 72 hours before embedding in paraffin wax blocks for histology. Before fixation, lungs were inflated using 1.5 ml of 4% PFA administered by intratracheal catheter (Exel International). An independent female cohort of 6- to 7-week-old female golden Syrian hamsters was also obtained from Charles River Laboratories and treated in an analogous manner. These hamsters were euthanized at 24 dpi, and collected tissues were processed in an identical manner. All animal experiments were performed according to protocols approved by the IACUC and Institutional Biosafety Committee at ISMMS and NYULH. Hamsters were randomly assigned to the different treatment groups, and all IAV and SARS-CoV-2 infections were performed in the BSL-3 facility.
Quantitative reverse transcription polymerase chain reaction
RNA was isolated from homogenized samples by TRizol/phenol-chloroform extraction. One microgram of total RNA from each tissue was reverse-transcribed into cDNA with oligo(dT) primers using SuperScript II reverse transcriptase (Thermo Fisher Scientific). qRT-PCR was performed using primers described in table S3 and KAPA SYBR Fast qPCR Master Mix (KAPA Biosystems) on LightCycler 480 Instrument II (Roche). Delta-delta-cycle threshold (DDCt) was determined relative to mock-infected control unless otherwise stated (21).

Hamster RNA-seq
RNA was isolated from homogenized samples by TRizol/phenol-chloroform extraction. One microgram of total RNA from each tissue was enriched for polyadenylated RNA and prepared for next-generation sequencing using the TruSeq Stranded mRNA Library Prep Kit (Illumina) according to the manufacturer’s instructions. Prepared libraries were sequenced on an Illumina NextSeq 500 platform. Fastq files were generated with bcftools (Illumina) and aligned to the golden Syrian hamster genome (Mes Aur 1.0, ensemble) using the RNA-seq Alignment application (Basespace, Illumina). Salmon files were analyzed using the DESeq2 analysis pipeline (101). All genes with $P_{adj} < 0.1$ were considered DEGs. GSEA studies were performed using the GSEA_4.1.0 Mac App as made available by the Broad Institute and the University of California, San Diego (102, 103). Analyses were conducted on a preranked gene list, with ranking statistic calculated from DESeq2 results output as follows: $–\log_{10}(P \text{ value}) \times \text{sign} (\log_{10}(FC))$ (104). Unbiased GSEAs were conducted against the Hallmark Gene Sets (v7.4), the curated C5 gene ontology and human phenotype ontology gene set (v7.4), and the curated C8 cell type signature gene sets (v7.4) made available by the Molecular Signatures Database (MSigDB). Additional GSEAs were conducted on gene sets manually curated from previous publications as described in the text. Normalized enrichment score and FDR q values (FDR values adjusted for analysis size) generated by these analyses were visualized using the ggplot2 package. All visualizations of RNA-seq differential expression data were created in R using ggplot2, pheatmap, ComplexHeatmap, and gplots packages. Gene set enrichment plots were adapted from VisualizeRNAseq (https://github.com/GryderArt/VisualizeRNAseq). Radar plots were created using the ggridar2 package (https://github.com/xl0418/ggridar2). Assessment of read coverage of viral genome was conducted using Bowtie2 and IGV_2.8.13 and visualized using ggplot2. Rank-rank scatterplots were created using the RHRO package using the same ranking statistic as was used in GSEAs (105).

H&E, Verhoeff Van Gieson, and TUNEL staining and quantification
Paraffin-embedded tissue blocks were cut into 5-µm sections and mounted on charged glass slides. Sections were deparaffinized by immersion in xylene and rehydrated in decreasing ethanol dilutions. Slides were then stained with hematoxylin (Gill’s formula, Vector Laboratories, catalog no. H3401) and eosin Y (Sigma-Aldrich, catalog no. E4009) according to the manufacturer’s instructions. Slides were dehydrated by immersion in increasing concentrations of ethanol, cleared with xylene, and coverslipped (21). Sections were assessed for clinical features by a board-certified pathologist. Images were morphometrically analyzed using QuPath (106) and ImageJ (107). Randomly sampled tissue regions were generated from digitized lung and kidney histological images. In kidneys, these regions were assessed for average cellular size across each area. In lung sampled areas, lambertosis coverage and airway sizes were manually quantified by treatment-blinded team members. Verhoeff Van Gieson staining was performed on 5-µm sections that were cut from paraffin-embedded tissue blocks and embedded on charged glass slides. Slides were stained using “Elastic Stain Kit (Verhoeff Van Gieson/EVG Stain)” (Abcam, ab150667) according to the manufacturer’s instructions. Slides were dehydrated by immersion in increasing concentrations of ethanol (Thermo Fisher Scientific), cleared with xylene (Thermo Fisher Scientific), and coverslipped (21). Slides were digitized using Hamamatsu S210 digital slide scanner. All images of slides were captured using NDP.view.2 software (Hamamatsu).

TUNEL staining was performed on 5-µm sections that were cut from paraffin-embedded tissue blocks and embedded on charged glass slides. Slides were deparaffinized and processed using the “TUNEL Assay Kit–BrdU-Red” kit (Abcam, ab666110) according to the manufacturer’s instructions. Nuclei were additionally stained with 4’,6-diamidino-2-phenylindole (DAPI). Slides were coverslipped and assessed by confocal microscopy. The total number of TUNEL* nuclei per cross section of tissue was manually tabulated.

Immunohistochemistry
Paraffin-embedded tissue blocks were cut into 5-µm sections and mounted on charged glass slides. Sections were deparaffinized by immersion in xylene and rehydrated in decreasing ethanol dilutions. Antigen retrieval was performed for 45 min in the IHC-Tek Epitope Retrieval Steamer (catalog no. IW-1102) with slides immersed in the IHC-Tek Epitope Retrieval Solution (catalog no. IW-1100). Tissue was blocked in tris-buffered saline (TBS) (Fisher Scientific) with 10% goat serum (MilliporeSigma) and 1% bovine serum albumin. Primary antibodies (MX-A: MilliporeSigma, MABF938; IBA1: Wako, 019-19741; CD3: Dako, A0452; MPO: Dako, A0398) were added to slides at the following dilutions: MX-A: 1:100; IBA1: 1:2500; CD3: 1:1000; and MPO: 1:5000. Sections were incubated overnight at 4°C. Slides were washed in TBS with 0.025% Triton X-100 (Thermo Fisher Scientific) before immersion in 0.3% hydrogen peroxide (Thermo Fisher Scientific) in TBS for 15 min. Slides were washed once again, and horseradish peroxidase–conjugated secondary antibody was added at a 1:5000 concentration (goat anti-mouse: Thermo Fisher Scientific, catalog no. A21426; goat anti-rabbit: Abcam, Ab6721). Slides were washed twice before application of 3,3′-diaminobenzidine (DAB) developing reagent (Vector Laboratories, catalog no. SK-4105). Slides were dehydrated by immersion in increasing concentrations of ethanol, cleared with xylene, and coverslipped. Slides were digitized using a Hamamatsu S210 digital slide scanner. All images of slides were captured using NDP.view.2 software (Hamamatsu).

Olfaction assessment
Olfaction was assessed using the buried food finding test as previously described (34, 108). Hamsters were presented with cereal (Coco Krispies, Kellogg’s) 5 days before the test; all were consumed within 1 hour. Twenty hours before testing, hamsters were food-restricted. On the day of testing, hamsters were placed into clean cages with standard bedding and allowed to acclimate for 20 min. After 20 min, hamsters were moved to a holding cage for 2 min, while chocolate cereal was buried underneath the bedding in a corner of the testing cage. Hamsters were then moved back to the cage with cereal in it and placed in the opposite corner of the cage as the buried cereal.
Hamsters were timed from placement in cage to the time of detection of food (digging in the area of the buried cereal). Hamsters were limited to a 15-min maximum period to find cereal. Once food was found, hamsters were moved back to holding cage for 1 min, and food was placed on top of the bedding (visible) in a corner of the test cage during this time. The hamster was then once again placed in the opposite corner of the test cage from the cereal, and time was recorded from placement of hamster in the cage to detection of food. All behavioral studies were in compliance with IACUC protocols and took place inside of a biosafety cabinet according to BSL-3 protocols.

**Marble burying assay**

The marble burying assay was adapted from previously described protocols (64). Hamsters were placed into a corner of a cage with clean bedding that had 20 equally spaced glass marbles placed inside of it. Hamsters were allowed to move freely about the cage for 15 min, at which time they were moved back to their original cage. The number of buried and unburied marbles per cage was tallied by two independent observers and averaged. Partially buried marbles were counted as buried if more than 60% of the marble was covered with bedding material. All behavioral studies were in compliance with IACUC protocols and took place inside of a biosafety cabinet according to BSL-3 protocols.

**RNA fluorescent in situ hybridization (RNAscope)**

The Fluorescent Multiplex V2 kit (Advanced Cell Diagnostics) was used for RNAscope. Specifically, we used the formalin-fixed, paraffin-embedded (FFPE) protocol as detailed in the RNAscope Multiplex Fluorescent Reagent Kit v2 Assay User Manual. RNAscope probes were as follows: Rbfox3 (NeuN) for pan-neuronal labeling (Mau-Rbfox3-C1) and the spike gene (S) for SARS-CoV-2 labeling (V-nCoV2019-S-C3). Opal dyes (Akoya Biosciences) were used for secondary staining as follows: Opal 690 for C1 and Opal 570 for C3. DAPI was used for nuclear staining. Images were taken on an LSM880 confocal microscope (Zeiss) with identical parameters between mock- and SARS-CoV-2–infected samples.

**Heart, lung, and kidney human sample collection**

All autopsies are performed with consent of next of kin and permitted for retention and research use of tissue. Autopsies were performed in a negative pressure room with protective equipment including N-95 masks; brain and bone were not obtained for safety reasons. All fresh tissues were procured before fixation and directly into TRIzol for downstream RNA extraction. Tissues were collected from the lung, kidney, and heart as consent permitted. Postmortem intervals ranged from less than 24 hours to 72 hours (with two exceptions, one at 4 days and one at 7 days, but passing RNA quality metrics) with an average of 2.5 days. All deceased patient remains were refrigerated at 4°C before autopsy.

**Human heart, lung, and kidney RNA-seq**

For RNA library preparation, all samples’ RNAs were treated with deoxyribonuclease (DNase) 1 (Zymo Research, catalog no. E1010). Post-DNase digested samples were then put into NEBNext rRNA depletion v2 (Human/Mouse/Rat), Ultra II Directional RNA (10 ng), and Unique Dual Index Primer Pairs were used following the vendor protocols from New England Biolabs. Completed libraries were quantified by Qubit and run on Bioanalyzer for size determination. Libraries were pooled and sent to the WCM Genomics Core or HudsonAlpha for final quantification using a Qubit fluorometer (Thermo Fisher Scientific), TapeStation 2200 (Agilent), and qRT-PCR using the Kapa Biosystems Illumina library quantification kit.

New York Genome Center (NYGC) RNA-seq libraries were prepared using the KAPA Hyper Library Preparation Kit + RiboErase, HMR (Roche) in accordance with the manufacturer’s recommendations. Briefly, 50 to 200 ng of total RNA were used for ribosomal depletion and fragmentation. Depleted RNA underwent first- and second-strand cDNA synthesis followed by adenylation and ligation of unique dual indexed adapters. Libraries were amplified using 12 cycles of PCR and cleaned up by magnetic bead purification. Final libraries were quantified using fluorescent-based assays including PicoGreen (Life Technologies) or Qubit fluorometer (Invitrogen) and Fragment Analyzer (Advanced Analytics) and sequenced on a NovaSeq 6000 sequencer (v1 chemistry) with 2 × 150 base pairs, targeting 60 million reads per sample.

RNA-seq data were processed through the nf-core/rnaseq pipeline (109). This workflow involved quality control of the reads with FastQC (www.bioinformatics.babraham.ac.uk) adapter trimming using TrimGalore! (https://github.com/FelixKrueger/TrimGalore), read alignment with STAR (110), gene quantification with Salmon (111), duplicate read marking with Picard MarkDuplicates (https://github.com/broadinstitute/picard), and transcript quantification with StringTie (112). Other quality control measures included RSeQC, Qualimap, and dupRadar. Alignment was performed using the GRCh38 build native to nf-core, and annotation was performed using Gencode Human Release 33 (GRCh38.p13). Differential expression comparisons were done as either COVID high cases versus COVID-negative controls or COVID low cases versus COVID-negative controls for each tissue specifically. SARS-CoV-2 viral load designations were assigned after quantification of normalized reads mapping to the SARS-CoV-2 genome for each donor. Genes were ranked by the following statistic: $\log_{10}(P \text{ value}) \times \log_{2}(\text{FoldChange})$. Ranked genes were used as input for GSEA on the MSigDB.

**Heart, lung, and kidney human sample collection**

Brain tissue and nasal epithelium, including the OE and OB, were retrieved under a collaborative effort by the Department of Neuro-pathology and the Department of Otolaryngology at Columbia University Irving Medical Center. The study was approved by the ethics and IRB of Columbia University Medical Center (IRB AAAT0689 and AAAS7370). Nasal tissues, including OE and respiratory epithelium, were harvested from the skull base using an en bloc resection of the anterior skull base including the cribriform plate. OE tissue was isolated from the olfactory cleft, spanning the turbinate and adjacent septal mucosa before being preserved in TRIzol reagent.

For human OE and OB, RNA was extracted from 10 mg of tissue per sample using Direct-zol RNA kit from Zymo Research (catalog no. R2052). After DNase treatment, 50 ng to 1 μg of total RNA were used to prepare DNA libraries with TruSeq RNA Library Prep Kit v2 (Illumina) following the manufacturer’s instruction. Libraries were amplified using 14 PCR cycles followed by AMPure XP bead purification. Next, libraries were quantified with Bioanalyzer (Agilent Technologies) and Qubit (Invitrogen) and sequenced on a NovaSeq 6000 sequencer (Illumina) at Columbia Genome Center.

All resulting fastq files were aligned to the *Homo sapiens* genome (GRCh38, RefSeq) using the RNA-seq alignment application.
SUPPLEMENTARY MATERIALS

and assessed for enrichment of various gene sets, as described in legends. Significance for these analyses was determined using statistical analyses, box and bar graphs, and Kaplan-Meier

Statistical analysis

Raw, individual-level data are presented in data file S1. All non–RNA-seq statistical analyses, box and bar graphs, and Kaplan-Meier plots were prepared using GraphPad Prism 9 as described in figure legends. Significance for these analyses was determined using statistical testing including analysis of variance (ANOVA) with post hoc analyses. Specific post hoc analyses and statistical thresholds are described in figure legends. In the marble burying test, all groups were assessed for outliers, which were corrected for using the iterative Grubb’s method. RNA-seq analysis was conducted using DESeq2. DEGs were defined as any genes with $P_{adj} < 0.1$. GSEA was performed as described in Materials and Methods; briefly, genes were ranked on the basis of $-\log_2(P\ value)/\text{sign}(\log_2[\text{Fold Change}])$ and assessed for enrichment of various gene sets, as described in both figure legends and the text using the Macos GSEA application (v4.1.0).

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S1

Tables S1 to S3

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