

# Neuropathological assessment of the olfactory bulb and tract in individuals with COVID-19

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## Summary

The majority of patients with Parkinson disease (PD) experience a loss in their sense of smell and accumulate insoluble  $\alpha$ -synuclein aggregates in their olfactory bulbs (OB). Subjects affected by a SARS-CoV-2-linked illness (COVID-19) frequently experience hyposmia. We previously hypothesized that  $\alpha$ -synuclein and tau misprocessing could occur following host responses to microbial triggers. Using semiquantitative measurements of immunohistochemical signals, we examined OB and olfactory tract specimens collected serially at autopsies between 2020 and 2023. Deceased subjects comprised 50 adults, which included COVID19+ patients (n=22), individuals with Lewy body disease (e.g., PD and dementia with Lewy bodies (DLB; n=6)), Alzheimer disease (AD; n=3), other non-synucleinopathy-linked degenerative diseases (e.g., progressive supranuclear palsy (PSP; n=2) and multisystem atrophy (MSA; n=1)). Further, we included neurologically healthy controls (HCO; n=9) and those with an inflammation-rich brain disorder as neurological controls (NCO; n=7). When probing for inflammatory changes focusing on anterior olfactory nuclei (AON) using anti-CD68 immunostaining, scores were consistently elevated in NCO and AD cases. In contrast, inflammation on average was not significantly altered in COVID19+ patients relative to controls, although anti-CD68 reactivity in their OB and tracts declined with progression in age. Mild-to-moderate increases in phospho- $\alpha$ Syn and phospho-tau signals were detected in the AON of tauopathy- and synucleinopathy-afflicted brains, respectively, consistent with mixed pathology, as described by others. Lastly, when both sides were available for comparison in our case series, we saw no asymmetry in the degree of pathology of the left versus right OB and tracts. We concluded from our autopsy series that after a fatal course of COVID-19, microscopic changes -when present- in the rostral, intracranial portion of the olfactory circuitry generally reflected neurodegenerative processes seen elsewhere in the brain. In general, inflammation correlated best with the degree of Alzheimer's-linked tauopathy and declined with progression of age in COVID19+ patients.

**Keywords:** COVID-19; olfaction; hyposmia; neurodegeneration; inflammation; amyloidosis; tauopathy; synucleinopathy, anterior olfactory nucleus

## Introduction

Parkinson disease (PD) is traditionally characterized clinically by extrapyramidal motor dysfunction and pathologically by the progressive degeneration of dopamine producing neurons in the substantia nigra. Over the past two decades, this nigral- and moto-centric view of PD has undergone a revision with the recognition that the disorder is also characterized by a wide variety of non-motor symptoms referable not only to brain pathology but to the peripheral nervous system as well [1]. Indeed, these symptoms may predate the onset of extrapyramidal motor dysfunction by decades [1]. Accordingly, a histopathological hallmark of typical, late-onset PD, namely intracellular  $\alpha$ -synuclein ( $\alpha$ Syn) aggregation in the form of Lewy bodies (LBs) and Lewy neurites (LNs), has been described in extra-nigral sites within the brain as well as within peripheral organs, often prior to their emergence in the substantia nigra. Braak and Del Tredici [2] provided pathological evidence for a dynamic spatiotemporal sequence of  $\alpha$ Syn pathology in the brain. According to their hypothesis, the OBs and dorsal motor nuclei of the vagus nerve represent the earliest CNS sites in which  $\alpha$ Syn aggregation occurs. Implicit in the Del Tredici-Braak model is the phenomenon of ‘spreading’ pathology, whereby -once initiated at those sites- the aggregation process is transmitted trans-synaptically to other nuclei including the substantia nigra, resulting in the well-recognized motor features of the disease [3]. Consistent with this hypothesis, hyposmia is one of the earliest and most frequent non-motor signs of PD [1,4], which accords with the presence of Lewy pathology in the OBs as well as in higher order olfactory structures [2]. As a potential trigger of  $\alpha$ Syn aggregation in the olfactory system and enteric nervous system, Braak and Del Tredici invoked an infectious, possibly viral, pathogen [3]. In this context, their hypothesis is compatible with the growing body of evidence implicating microbial encounters as risk factors for neurodegenerative disorders in later years (reviewed in [5]).

Subsequent to its discovery in Wuhan, China in late 2019, coronavirus-linked disease 2019 (COVID-19), caused by severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), spread rapidly throughout the world, culminating in a global pandemic that imposed substantial human suffering and loss of life as well as unprecedented social and economic consequences [6]. Olfactory dysfunction in the form of anosmia, hyposmia, and parosmia were described as a common symptom during the pandemic, affecting 30–70% of all patients with confirmed COVID-19 [7]. Abnormal olfaction usually occurs early in the course of infection, presenting as the first

symptom in approximately 12% of all patients [8]. Although most patients recovered their sense of smell gradually, often within 3 to 4 weeks [9,10], some suffered from persistent impairment, suggesting severe and/or permanent damage to components within the olfactory circuitry [9]. Several possible processes have been invoked as mechanisms underlying olfactory dysfunction in COVID-19, including direct infection of olfactory sensory neurons (OSNs) in the nasal cavity [11] as well as primary infection of sustentacular cells with secondary injury to OSNs. In accordance with the latter, several investigators have noted the absence of the SARS-CoV-2 target receptor, angiotensin converting enzyme-2, on neuronal cells, and instead proposed infection and damage to non-neuronal support cells in the olfactory epithelium [12,13]. Regardless of the cellular target, there is histopathological evidence that infection by SARS-CoV-2 can confer effects onto the CNS in the form of local inflammation, axonal pathology and microvascular changes in the OB and olfactory tracts (OT) [14].

Moreover, a recent population-based neuroimaging study in survivors of a SARS-CoV-2 infection revealed chronically reduced volumes of olfaction circuitry-associated areas in the brain. It provided evidence that the potential impact of SARS-CoV-2 on scent processing extends beyond the OT to impose lasting neurodegenerative and/or remodeling changes in the central olfactory circuitry [15].

That COVID-19 and PD share olfactory dysfunction as an early clinical sign is intriguing in the context of the Braak-Del Tredici hypothesis, which implicates exposure to one or several pathogens in disease initiation. Moreover, the presence of  $\alpha$ Syn in the mammalian olfactory epithelium and mucosa [16] renders the nasal passages a plausible site for a disease-initiating interaction between airborne pathogens, such as RNA viruses, and  $\alpha$ Syn, a highly abundant brain protein that is prone to misfolding. Consistent with this possibility, animal studies have revealed an upregulation and accumulation of  $\alpha$ Syn in response to intranasal SARS-CoV2 administration [17,18]. It is believed that the impact of the virus on  $\alpha$ Syn metabolism may be imposed either via a direct interaction intracellularly or may be related to innate, immunomodulatory functions of extracellular  $\alpha$ Syn [19]. With respect to the former, heparin-binding sites on the SARS-CoV-2 spike protein may act as facilitator to seed the aggregation of other heparin-binding proteins, including  $\alpha$ Syn, as well as amyloid  $\beta$ -peptide and tau [20]. Further, SARS-CoV-2 proteins are

capable of forming amyloid aggregates themselves [21], possibly providing a nidus for the aggregation of neurodegeneration-associated proteins. Alternatively, or in addition,  $\alpha$ Syn has been demonstrated to function as an innate, anti-viral factor [16,22]. In theory, its upregulation in response to SARS-CoV2 infection could predispose it to aggregation, thereby putting COVID-19 patients at higher risk of developing PD-relevant changes [23]. Of note, few cases of parkinsonism associated with SARS-CoV-2 infection in humans have been documented since the beginning of the pandemic [24–26]. In contrast, a substantial number of patients with established PD experienced worsening of their symptoms during and after recovery from COVID-19 illness [27].

These lines of evidence implicate SARS-CoV-2 infection as a possible trigger for the initiation of PD-linked pathology. However, the scarcity of histopathological studies interrogating inflammatory and neurodegenerative changes and specifically probing for  $\alpha$ Syn aggregation in the olfactory pathway following SARS-CoV-2 infection, represents a gap in our understanding of the relationship between the two disorders. In the present autopsy study, we employ an immunohistochemical approach to document the nature and extent of inflammation and of neurodegenerative changes within rostral olfactory structures, specifically the OB and OT, among a cohort of individuals that died of COVID-19-related complications versus those with neurodegenerative synucleinopathies, including PD, dementia with Lewy bodies (DLB), and multisystem atrophy (MSA). In addition, in light of reports that COVID-19 may predispose to Alzheimer disease (AD) [28], we have included participants with dementia as well as another tauopathy, progressive supranuclear palsy (PSP). We compared inflammatory and neurodegenerative changes in the OB and OT of these groups with a series of COVID-19-negative subjects without a neurodegenerative disease. Among the latter, we included as a further control a subset of participants with primary inflammatory disorders of the brain.

## Methods

### *Study Subjects*

Study subjects were recruited at two sites: The Ottawa Hospital in Ottawa, Ontario, Canada and the Kantonsspital St. Gallen, in St. Gallen, Switzerland. Ethics approval was obtained from the Ottawa Health Science Network Research Ethics Board (#20120963-01H) and the Kantonsspital St. Gallen (Ethikkommission Ostschweiz, Projekt-ID 2021-00678) respectively.

Characteristics of the study groups are summarized in **Table 1**. We examined tissue from 50 individuals: 16 COVID-19-negative controls, including 9 neurologically healthy participants (HCO) and 7 with inflammatory CNS disorders (referred to as neurological controls, NCO), 22 subjects who died from SARS-CoV-2 infection-related complications (referred to as COVID19+), 6 participants with Lewy Body disease (LBD), three with Alzheimer disease (AD), and three with other neurodegenerative diseases (OND; progressive supranuclear palsy (PSP), n=2; multisystem atrophy (MSA), n=1. The age of subjects ranged from 51 to 90 years. All COVID19+ patients were clinically free of a previously identified neurodegenerative illness based on medical chart review during their admission to the hospital, with the exception of one person, who demonstrated signs of parkinsonism, but without a formal diagnosis by a neurologist. Of note, olfactory function was not routinely measured in study participants; one COVID19+ patient reported the loss of sense of smell on admission. Details with respect to causes of death, clinical diagnoses and a summary of the sample inventory can be found in **Supplemental Table 1**.

#### *Tissue processing*

Tissues were removed and processed in the Departments of Pathology at The Ottawa Hospital and the Kantonsspital St. Gallen according to routine procedures for *post mortem* brain collection. Following their removal at autopsy, brain specimens were fixed typically for 10-14 days, rarely as long as 42 days, in 20% neutral buffered formalin. Following fixation, OBs and OT were dissected from the brain (unless they had already been separated at the time of removal of the rest of the brain) and embedded in paraffin.

#### *Immunohistochemistry*

Sections were cut at 5  $\mu$ m and mounted onto coated slides. Slides were deparaffinized in Citrisolv and rehydrated through a series of decreasing ethanol concentrations. Endogenous peroxidase activity was quenched with 0.3% hydrogen peroxide in methanol, followed by heat-induced antigen retrieval with citrate buffer, pH 6.0. For amyloid- $\beta$  peptide staining, slides were also incubated in 98% formic acid for 5 minutes at room temperature. To reduce non-specific binding, sections were blocked in 10% goat serum in PBS-T (PBS + 0.1% Triton-X-100 + 0.05% Tween-20) for 30 minutes at room temperature. Sections were incubated overnight at 4 °C in primary antibodies diluted in 5% goat serum in PBS-T. Primary antibodies included those to: p- $\alpha$ Syn,

pSyn#64 (Wako, cat# 015-25191, 1:500); LB509 (Biolegend, cat# 807702, 1:10,000); amyloid- $\beta$  peptide, 6E10 (Biolegend cat# 803001, 1:1000); KiM1P (CD68; prepared in the Stadelmann lab; Radzun et al. 1991 [29]; 1:50); and SARS Nucleocapsid protein (Novus, cat# NB100-56576, 1:250). Biotinylated, secondary antibody anti-mouse or anti-rabbit IgG (H + L), made in goat (Vector Labs, BA-9200 or BA-1000) was diluted to 1:225, and sections were incubated for 1 hr at room temperature. The signal was amplified with VECTASTAIN® Elite® ABC HRP Kit (Vector Labs, PK-6100) for 1 hr at room temperature and visualized using 3'3-diaminobenzidine (Sigma, SIGMAFAST™ DAB, D4293). Samples were counterstained with Harris' Modified Hematoxylin stain and dehydrated through a series of increasing ethanol concentration solutions and Citrisolv. Permount Mounting Medium (Fisher Scientific, SP15-100) was used for mounting, and developed slides were dried and scanned for visualization. The detailed IHC protocol can be found here: [dx.doi.org/10.17504/protocols.io.kqdg3p7mql25/v1](https://dx.doi.org/10.17504/protocols.io.kqdg3p7mql25/v1)

Immunohistochemical staining for p-tau was performed in the Louise Pelletier Histopathology Core Facility in The Department of Pathology and Laboratory Medicine at The University of Ottawa using the Bond Polymer Refine Detection Kit (DS9800) with the Leica Bond™ system. Sections were deparaffinized and incubated using a 1:2500 dilution of mouse p-tau antibody (#MN1020, AT8 clone; ThermoFisher) for 15 minutes at room temperature and detected using an HRP conjugated compact polymer system. Slides were then stained using DAB as the chromogen, counterstained with Hematoxylin, mounted and cover slipped.

Image visualization across sites was performed using an *in-house* Omero Server software 5.6.6 [30] and immunohistochemistry figures were created using an *in-house* Omero Figure software v6.0.0.

Additional details of reagents can be found in **Supplemental Table 2**.

### *Quantification*

One section from each OB/OT was stained and analysed for each marker. In preliminary studies, it was evident that staining for neurodegenerative proteins was confined predominantly to the anterior olfactory nuclei (AON); thus, semi-quantitative scoring of immunoreactivity and intensity of inflammatory as well as neurodegenerative changes were focused on these nuclei, visually defined as groups of larger neurons lying on a neuropilic background (**Figure 1**). One section for

each bulb was stained for CD68 reactivity as a surrogate marker for inflammation. For CD68 and neurodegenerative immunostaining, semi-quantitative analyses were performed using a scale from 0 to 5, corresponding to increasing densities of DAB-positive reactivity. **Supplemental Tables 3-7** indicate the number of AONs analyzed in each section, which tissue region was available for each case, as well as the score assigned for each marker used.

#### *Statistical analysis*

Statistical analyses were performed using GraphPad Prism version 10 (GraphPad Software [www.graphpad.com](http://www.graphpad.com)). Differences between groups were determined using the Kruskall-Wallis test followed by Dunn's post-hoc analysis. Correlation between staining score and age was determined using Pearson's correlation tool. For all statistical analyses, a cut-off for significance was set at 0.05. Data are displayed with p values represented as \*p < 0.05, \*\*p < 0.01, \*\*\*p < 0.001, and \*\*\*\*p < 0.0001.

## Results

Hematoxylin and eosin (HE)-stained sections of the OB and attached OT are shown in **Figures 1A and B**. These are representative of all tissue sections used in the analyses, and they indicate the location and appearance of the AON. As expected, in cases from older individuals, abundant corpora amylacea were often observed along white matter structures within the OT and were concentrated in the subpial neuropil (**Fig 1C**).

When we tested for SARS-CoV2 reactivity in sections of COVID19+ patients in our autopsy series using a specific antibody against its nucleocapsid protein, we did not detect any signal for the viral protein in any of the OB and OT sections analyzed under these conditions. In contrast, the same antibody readily detected the nucleocapsid protein in sustentacular cells of the olfactory epithelium in mice nasally inoculated with a mouse-adapted variant of SARS-CoV2 (not shown) [31].

The degree of tissue inflammation was assessed using anti-CD68 immunoreactivity for the detection of histiocytes and microglia. Positive CD68-immunoreactive cells were observed throughout the OB and OT (**Fig 2A**). To correlate inflammatory signals with neurodegenerative changes, we focused our semi-quantitative analysis of CD68 reactivity on the AON. As indicated

in **Fig 2C**, all autopsy cases exhibited some degree of positive staining (a score of 1, or higher), regardless of disease status. For the purpose of comparing inflammatory changes, we divided the non-neurodegenerative controls into healthy controls (HCO) and those whose diagnosis at autopsy involved an inflammatory condition of the CNS (neurological controls; NCO). Because on average there was no significant difference in CD68 reactivity scores between HCO and NCO brains, these brains were grouped together as controls in subsequent analyses. Sections of AD brains generated the highest average inflammation score (4.7) in OB and OT, followed by the NCO group (average score, 4). COVID19+ cases had significantly lower CD68 staining scores than the NCO group (**Fig 2C**). Interestingly, inflammation scores based on CD68 staining correlated negatively ( $R=-0.4494$ ) with age in COVID19+ patients (**Fig 2D**).

Phosphorylated  $\alpha$ Syn (p- $\alpha$ Syn)-positive pathology was found to be restricted to the AON (**Fig 3A**). There, immunostaining was also scored on a semi-quantitative scale (as above) with representative images shown in **Fig 3B**. Not surprisingly, p- $\alpha$ Syn pathology was detected within the AON in all LBD cases (**Fig 3C**). Lewy neurites (LNs) and smaller, granular neuropil inclusions were found in several AON, but no definite, typical spherical Lewy bodies (LBs) within neuronal perikarya were observed. In the MSA case, abundant, pathognomonic glial cytoplasmic inclusions in oligodendrocytes were identified throughout the OB and OT (**Supp Fig 1**). Typical p- $\alpha$ Syn pathology was detected in four of 22 (18%) COVID19+ cases. Three subjects had no documented history of clinical signs of parkinsonism, whereas one was suspected to have parkinsonism (without a formal neurological diagnosis) (**Fig 3C**). In our series, anti-p- $\alpha$ Syn reactivity did not correlate with age (**Fig 3D**). Importantly, three of four anti-p- $\alpha$ Syn-positive COVID19+ subjects showed evidence of ‘incidental LBD’ in the form of LBs and LNs in the substantia nigra and dorsal motor nucleus of the vagus nerve. A fourth p- $\alpha$ Syn-positive COVID19+ case showed only a single reactive neurite in the OB, thus being assigned a score of 1. LBD cases were found to have a significantly higher average pathology score (4.3) than the control groups and COVID19+ cases (**Fig 3E**). One AD case with positive p- $\alpha$ Syn pathology staining was diagnosed with mixed pathology (AD plus LBD) when assessing the entire brain (**Fig 3C**). Immunohistochemical analyses using the anti- $\alpha$ Syn antibody, LB509, revealed similar results when compared to those using anti-p- $\alpha$ Syn (**Supp Fig 2**).

Phosphorylated tau (p-tau) inclusions were detected by the AT8 antibody in 90% of the cases and were confined to the AON (**Fig 4A**). These tau-immunoreactive aggregates included dystrophic neurites as well as neurofibrillary tangles. Representative images corresponding to the semi-quantitative scale used for scoring (as above) are shown in **Fig 4B**. There was no correlation between the severity of p-tau pathology and the age of neurologically healthy cases (**Fig 4D**). AD cases had the highest average pathology score (5.0) among all groups, which was significantly higher than reactivity seen in the control and COVID19+ groups. (**Fig 4E**).

When detected, amyloid- $\beta$  peptide-specific pathology was found to be restricted to the AON (**Fig 5A**), with representative images shown in **Fig 5B**. As expected, all cases in the AD group were found to have amyloid- $\beta$  pathology. A small percentage of control and COVID19+ cases were found to have amyloid- $\beta$  plaques in the AON, while other neurodegenerative cases did not show any detectable amyloid- $\beta$ -positive pathology in their OB or OT (**Fig 5C**). There was no correlation between the degree of amyloid- $\beta$  peptide burden and age in control or COVID19+ cases (**Fig 5D**). One NCO case (grouped here together with HCO) with amyloid- $\beta$ -related angiitis generated an amyloid- $\beta$  score of 3. The COVID19+ brain that contained the highest amyloid- $\beta$  peptide load showed AD neuropathologic changes on *post mortem* examination of other brain regions despite the absence of a documented clinical history of dementia. When looked at as a group, AD cases had a significantly higher average score for amyloid- $\beta$  deposition (4.7), as expected, than corresponding tissues from controls, COVID19+ and OND subjects (**Fig 5E**).

Lastly, we sought to correlate staining for neurodegenerative proteins with the degree of CD68-immunoreactivity (**Fig 6A-C**). When comparing the cases with a pathology score of 1 or above to those with no pathology (score 0), p-tau cases with a score of 5 had significantly higher CD68 reactivity (**Fig 6B**). Individual trends but no significant differences were seen when correlating anti-CD68 immunoreactivity scores with the degree of synucleinopathy or amyloid- $\beta$  peptide amyloidosis (**Fig 6A,C**).

Individual scores for the immunostaining with each antibody for each of the 50 cases are shown in **Table 2**, with additional details summarized in **Supplemental Tables 3-7**.

## Discussion

Previous authors have pointed at a possible association between SARS-CoV-2 infection and neurodegeneration, as identified by a rise in the incidence of cognitive decline and parkinsonism in survivors [28,32–34]. A recent imaging study of COVID19+ subjects, including those with mild disease, revealed reductions in grey matter thickness in olfaction-related structures as well as other regions functionally connected to the olfactory cortex [15]. In contrast, other investigators [35,36] reported increases in gray matter volumes in distinct anatomical regions. These divergent results likely reflect substantial complexity in the relationship between pathogenic SARS-CoV-2 variants, genetic susceptibility of the host, mechanisms of disease, and the period that has passed between infection and assessment of outcomes.

Here, we did not find any evidence for a relationship between the history of a recent COVID-19-related illness, which had warranted hospital admission and was ultimately fatal, and features of  $\alpha$ Syn pathology in the OB or AON, using immunohistochemical staining with two well-characterized, specific antibodies as a readout. Although we detected  $\alpha$ Syn aggregates within the AON in four of 22 (18%) of the neurologically intact COVID19+ group, three of these subjects showed typical Lewy pathology in the brainstem, with only one of those having had suspected parkinsonism as per the medical record. It is generally thought that these hallmark findings of PD are generated over a decade (or more) [37], whereas the course of COVID-19 illness that lead to death in our study subjects was on average two weeks following hospitalization (**Supplemental Table 1**). Therefore, we speculate that the observed pathology in these cases reflected pre-existing LBD that occurred independently of SARS-CoV-2 infection. In this respect, our results are compatible with those of Blanco-Palmero and co-workers, who observed no significant difference in total  $\alpha$ Syn levels in the cerebrospinal fluid or serum among COVID-19 patients versus HCO subjects [38].

However, our findings do not exclude an effect of the viral infection on subtle  $\alpha$ Syn misprocessing in the olfactory system, as it is conceivable that there was insufficient time for immunohistochemically detectable, fibrillar  $\alpha$ Syn aggregates to form. An implication of this caveat is that in the future more sensitive approaches for the detection of soluble oligomers, of post-translationally modified conformers, of in vitro seeding-competent species or of alternatively

spliced mRNA transcripts could reveal such an association, including in the early stages after COVID-19 infection.

Previous *post mortem* analyses of COVID-19 patients had revealed local inflammation associated with axonal pathology and microvascular changes in the OB and OT [14], as well as the finding of increased immune activation and microglial nodules, as reported for example by Schwabenland et al. [39]. In our study, the absence of significant inflammation in COVID19+ subjects relative to controls, as judged by using CD68 immunoreactivity as a readout, is consistent with findings by Matschke et al. [40]. In our case series and akin to the evidence regarding  $\alpha$ Syn aggregation, this relative paucity of inflammation within the OB and OT could also be related to the relatively short duration of illness, its transient nature, or lack of actual tissue invasion by virions. Conversely, one could speculate that less visible inflammation may reflect a relatively deficient immune response in older subjects, rendering them more susceptible to COVID-19 infection in general. Along these lines, we found that CD68 scores in the rostral olfactory circuitry were negatively associated with age in our COVID19+ cases, possibly reflecting a decline in anti-viral responses. Studies are underway to examine the degree of inflammation in the piriform cortex, to which the olfactory tracts project.

One shortcoming of our study is that no quantification of olfactory function had been performed in any of the participants that subsequently died within the hospital, in large part due to the severity of respiratory distress that warranted intubation. In a parallel effort, we are currently analyzing serial sections of the intact nasal cavity, including olfactory and respiratory epithelia, from all 50 study participants, where we will revisit the rate of detection of SARS-CoV-2 nucleocapsid proteins. When probing for the presence of viral proteins in OB and OT sections from three, PCR-confirmed COVID19+ cases in our series, we detected no specific evidence of a viral infection.

Although we were unable to procure evidence for a direct relationship between COVID-19 infection and olfactory  $\alpha$ Syn aggregation, our study revealed interesting findings of relevance to olfactory dysfunction in neurodegeneration and perhaps to ageing as well. For example, our demonstration that the overall severity of  $\alpha$ Syn as well as amyloid- $\beta$  and tau pathology of the OB and AON was highest in the LBD and AD cases, respectively, is consistent with the frequent association of hyposmia with these diseases. Conversely, the relative paucity of  $\alpha$ Syn and tau

pathology within AON neurons from our MSA and PSP subjects, respectively, correlates with the absence of a loss in sense of smell as a cardinal clinical manifestation in these diseases [41].

The existence of tau and  $\alpha$ Syn co-pathology in almost all of the synucleinopathy cases in our series suggests that the molecular mechanisms which underlie the frequent co-existence of these changes in the brains of AD and LBD patients are also operative in the intracranial olfactory circuitry.

Our rationale for including subjects with AD derived from evidence that SARS-CoV-2 infection may facilitate cognitive decline [28] and may disrupt both amyloid- $\beta$  and tau homeostasis, leading to tau hyperphosphorylation, as suggested in part by elevated plasma concentrations of both total and phosphorylated tau in living subjects [42–45]. We detected no strong evidence yet to link COVID-19 infection to  $\beta$ -amyloidosis in the rostral olfactory circuitry. However, three COVID19+ cases (13.6%) compared to a single control individual (6.25%) were found to have amyloid- $\beta$  pathology in the OB and OT. Although the numbers of cases in each diagnostic group are too small to draw definitive conclusions, this result is intriguing considering studies that showed SARS-CoV-2, herpetiform DNA viruses and bacteria can alter amyloid- $\beta$  homeostasis and modify neuropathological outcomes [42,46].

We detected no evidence for a relationship between COVID-19 illness and p-tau deposition in the OB or AON at autopsy. Consistent with recent findings by Tremblay and colleagues [47], 90% of our cohort, including control participants, displayed at least some degree of tau pathology. This is perhaps not surprising given that the process of tau hyperphosphorylation and aggregation is not confined to primary neurodegenerative diseases. Indeed, changes in tau metabolism are increasingly recognized as sequelae of diverse insults to the brain, including seizures, trauma, viral infections and autoimmune disease [48,49], and as a part of physiological ageing [47]. In this context, the prevalence of tau pathology in the OB and AON across our entire cohort may represent a morphological surrogate for a variety of insults, including pathogen-induced inflammation, sustained by individuals throughout their lifetime. Perhaps consistent with this, tauopathy cases in our case series showed the highest inflammation (anti-CD68 reactivity-based) scores (**Fig 6B**). However, assuming that p-tau aggregation and inflammation may be related, it is not possible to ascribe temporal precedence to one or the other due to the cross-sectional nature of our study.

In conclusion, we found no evidence that a subacute, fatal SARS-CoV-2 infection induces  $\alpha$ Syn aggregation in the OB or AON of human subjects under the microscopic conditions examined. However, our study has limitations and further investigations addressing these are required prior to excluding a role for this RNA virus in triggering neurodegeneration within the olfactory system. The most critical of these is the discrepancy between the duration of illness, which in our cohort was short, and the time required for neurodegenerative changes to be detectable by traditional immunohistochemistry. Related to this, the subjects in our cohort were all above 50 years of age, thereby complicating the attribution of neurodegenerative changes to SARS-CoV-2 alone versus those related to ageing, including those clinically not yet detected. Future studies should include more subjects, including younger individuals, those with a longer duration of viral illness and those with neurological signs as a result of their infectious illness. We will supplement histological analyses with more sensitive, biochemically based techniques and quantification of transcriptional changes to detect pathologically relevant structural and molecular alterations in the proteins of interest. In our prospectively collected cohort, there were no female subjects in our LBD and OND groups, precluding analysis of sex-related differences in these subsets. Further, because this post mortem study was cross-sectional, it precluded analyses of any temporal relationship(s) among the changes observed.

To address any long-term sequelae versus short-term effects of RNA virus infections of the upper respiratory tract on brain health, such as pertaining to  $\alpha$ Syn, tau and amyloid- $\beta$  peptide homeostasis, we have recently begun to conduct experiments in mice, including those that carry PD-linked allelic variants [16,50]. These ongoing studies in animal models include the inoculation of the nasal cavity with a mouse-adapted strain of SARS-CoV-2 [31] to monitor its potential aggregation effects on neurodegeneration-linked proteins. As mentioned, our investigation here was confined to the OB and AON. Autopsy studies interrogating the impact of SARS-CoV-2 infection in humans on  $\alpha$ Syn metabolism (and inflammation) at a more rostral site in the olfactory epithelium as well as more proximally in the piriform cortex are also currently ongoing. We anticipate that these will add to an ultimately anatomically more complete assessment of this potentially important environment-brain interaction.

## Supplementary Information

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### *Author Contributions*

NAL, JJT, JF, CSN, aSCENT-PD Investigators, JMW and MGS conceptualized the study; NAL, carried out the histological assessments; NAL, JJT, JMW and MGS analyzed data; AKJ, JF, CSN, LF, OH, WJ, JP, JMW collected autopsy material, NAL, JMW, JJT, MGS wrote the manuscript and all authors provided feedback on manuscript.

## References

1. Kalia LV, Lang AE. Parkinson's disease. *The Lancet*. 2015;386:896–912.
2. Braak H, Tredici KD, Rüb U, de Vos RAI, Jansen Steur ENH, Braak E. Staging of brain pathology related to sporadic Parkinson's disease. *Neurobiol Aging*. 2003;24:197–211.
3. Braak H, Rüb U, Gai WP, Del Tredici K. Idiopathic Parkinson's disease: possible routes by which vulnerable neuronal types may be subject to neuroinvasion by an unknown pathogen. *J Neural Transm Vienna Austria* 1996. 2003;110:517–36.
4. Li J, Mestre TA, Mollenhauer B, Frasier M, Tomlinson JJ, Trenkwalder C, et al. Evaluation of the PREDIGT score's performance in identifying newly diagnosed Parkinson's patients without motor examination. *Npj Park Dis*. 2022;8:1–12.
5. Smeyne RJ, Noyce AJ, Byrne M, Savica R, Marras C. Infection and Risk of Parkinson's Disease. *J Park Dis*. 2021;11:31–43.
6. Al-Aly Z, Xie Y, Bowe B. High-dimensional characterization of post-acute sequelae of COVID-19. *Nature*. 2021;594:259–64.
7. Agyeman AA, Chin KL, Landersdorfer CB, Liew D, Ofori-Asenso R. Smell and Taste Dysfunction in Patients With COVID-19: A Systematic Review and Meta-analysis. *Mayo Clin Proc*. 2020;95:1621–31.
8. Sayin İ, Yaşar KK, Yazıcı ZM. Taste and Smell Impairment in COVID-19: An AAO-HNS Anosmia Reporting Tool-Based Comparative Study. *Otolaryngol–Head Neck Surg Off J Am Acad Otolaryngol-Head Neck Surg*. 2020;163:473–9.
9. Lechien JR, Chiesa-Estomba CM, Beckers E, Mustin V, Ducarme M, Journe F, et al. Prevalence and 6-month recovery of olfactory dysfunction: a multicentre study of 1363 COVID-19 patients. *J Intern Med*. 2021;290:451–61.
10. Xydakis MS, Dehgani-Mobaraki P, Holbrook EH, Geisthoff UW, Bauer C, Hautefort C, et al. Smell and taste dysfunction in patients with COVID-19. *Lancet Infect Dis*. 2020;20:1015–6.
11. Meinhardt J, Radke J, Dittmayer C, Franz J, Thomas C, Mothes R, et al. Olfactory transmucosal SARS-CoV-2 invasion as a port of central nervous system entry in individuals with COVID-19. *Nat Neurosci*. 2021;24:168–75.
12. Khan M, Yoo S-J, Clijsters M, Backaert W, Vanstapel A, Speleman K, et al. Visualizing in deceased COVID-19 patients how SARS-CoV-2 attacks the respiratory and olfactory mucosae but spares the olfactory bulb. *Cell*. 2021;184:5932–5949.e15.
13. Zazhytska M, Kodra A, Hoagland DA, Frere J, Fullard JF, Shayya H, et al. Non-cell-autonomous disruption of nuclear architecture as a potential cause of COVID-19-induced anosmia. *Cell*. 2022;185:1052–1064.e12.

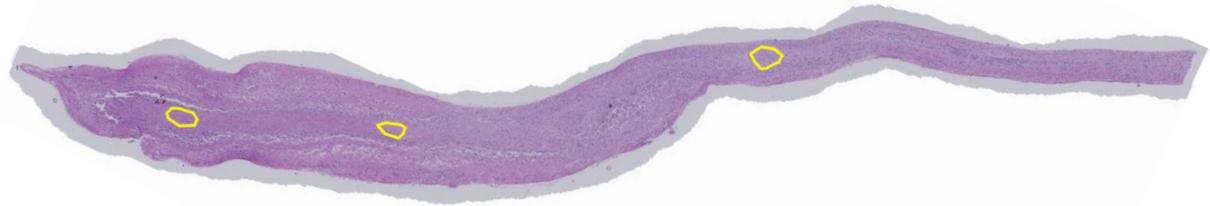
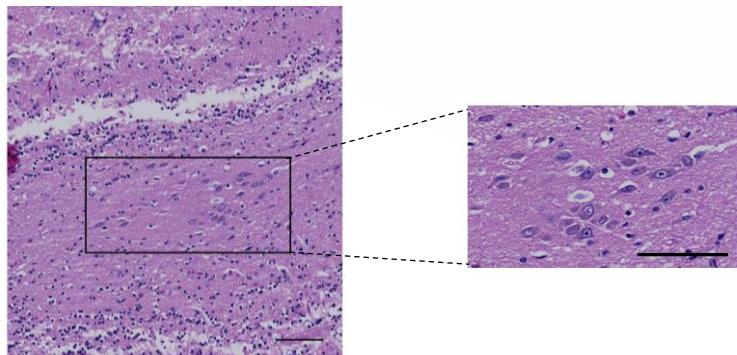
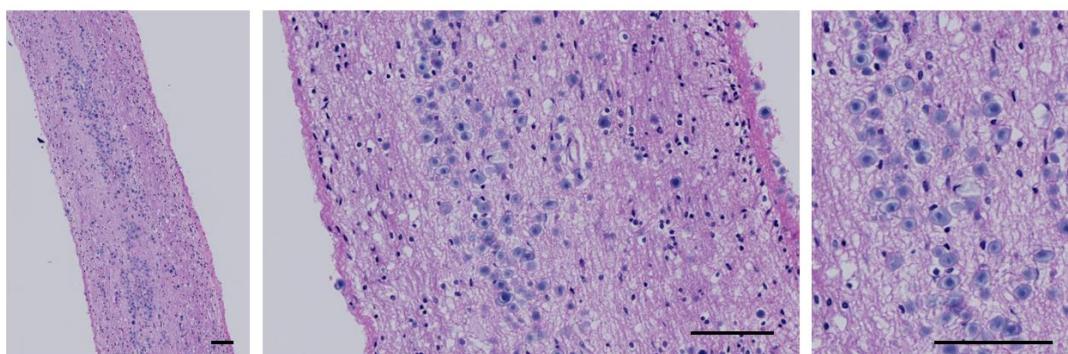
14. Ho C-Y, Salimian M, Hegert J, O'Brien J, Choi SG, Ames H, et al. Postmortem Assessment of Olfactory Tissue Degeneration and Microvasculopathy in Patients With COVID-19. *JAMA Neurol.* 2022;79:544–53.
15. Douaud G, Lee S, Alfaro-Almagro F, Arthofer C, Wang C, McCarthy P, et al. SARS-CoV-2 is associated with changes in brain structure in UK Biobank. *Nature.* 2022;1–17.
16. Tomlinson JJ, Shutinoski B, Dong L, Meng F, Elleithy D, Lengacher NA, et al. Holocranohistochemistry enables the visualization of  $\alpha$ -synuclein expression in the murine olfactory system and discovery of its systemic anti-microbial effects. *J Neural Transm.* 2017;124:721–38.
17. Käufer C, Schreiber CS, Hartke A-S, Denden I, Stanelle-Bertram S, Beck S, et al. Microgliosis and neuronal proteinopathy in brain persist beyond viral clearance in SARS-CoV-2 hamster model. *eBioMedicine* [Internet]. 2022 [cited 2023 Mar 30];79. Available from: [https://www.thelancet.com/journals/ebiom/article/PIIS2352-3964\(22\)00183-9/fulltext](https://www.thelancet.com/journals/ebiom/article/PIIS2352-3964(22)00183-9/fulltext)
18. Philippens IHCHM, Böszörényi KP, Wubben JAM, Fagrouch ZC, van Driel N, Mayenburg AQ, et al. Brain Inflammation and Intracellular  $\alpha$ -Synuclein Aggregates in Macaques after SARS-CoV-2 Infection. *Viruses.* 2022;14:776.
19. Iravanpour F, Farrokhi MR, Jafarinia M, Oliaee RT. The effect of SARS-CoV-2 on the development of Parkinson's disease: the role of  $\alpha$ -synuclein. *Hum Cell.* 2023;
20. Tavassoly O, Safavi F, Tavassoly I. Seeding Brain Protein Aggregation by SARS-CoV-2 as a Possible Long-Term Complication of COVID-19 Infection. *ACS Chem Neurosci.* 2020;11:3704–6.
21. Bhardwaj T, Gadhave K, Kapuganti SK, Kumar P, Brotzakis ZF, Saumya KU, et al. Amyloidogenic proteins in the SARS-CoV and SARS-CoV-2 proteomes. *Nat Commun.* 2023;14:945.
22. Beatman EL, Massey A, Shives KD, Burrack KS, Chamanian M, Morrison TE, et al. Alpha-Synuclein Expression Restricts RNA Viral Infections in the Brain. *J Virol.* 2015;90:2767–82.
23. Brundin P, Nath A, Beckham JD. Is COVID-19 a Perfect Storm for Parkinson's Disease? *Trends Neurosci.* 2020;43:931–3.
24. Cohen ME, Eichel R, Steiner-Birmanns B, Janah A, Ioshpa M, Bar-Shalom R, et al. A case of probable Parkinson's disease after SARS-CoV-2 infection. *Lancet Neurol.* 2020;19:804–5.
25. Faber I, Brandão PRP, Menegatti F, de Carvalho Bispo DD, Maluf FB, Cardoso F. Coronavirus Disease 2019 and Parkinsonism: A Non-post-encephalitic Case. *Mov Disord Off J Mov Disord Soc.* 2020;35:1721–2.
26. Méndez-Guerrero A, Laespada-García MI, Gómez-Grande A, Ruiz-Ortiz M, Blanco-Palmero VA, Azcarate-Díaz FJ, et al. Acute hypokinetic-rigid syndrome following SARS-CoV-2 infection. *Neurology.* 2020;95:e2109–18.

27. Leta V, Rodríguez-Violante M, Abundes A, Rukavina K, Teo JT, Falup-Pecurariu C, et al. Parkinson's Disease and Post-COVID-19 Syndrome: The Parkinson's Long-COVID Spectrum. *Mov Disord Off J Mov Disord Soc*. 2021;36:1287–9.
28. Wang L, Davis PB, Volkow ND, Berger NA, Kaelber DC, Xu R. Association of COVID-19 with New-Onset Alzheimer's Disease. *J Alzheimers Dis*. 2022;89:411–4.
29. Radzun HJ, Hansmann ML, Heidebrecht HJ, Bödewadt-Radzun S, Wacker HH, Kreipe H, et al. Detection of a monocyte/macrophage differentiation antigen in routinely processed paraffin-embedded tissues by monoclonal antibody Ki-M1P. *Lab Investig J Tech Methods Pathol*. 1991;65:306–15.
30. Allan C, Burel J-M, Moore J, Blackburn C, Linkert M, Loynton S, et al. OMERO: flexible, model-driven data management for experimental biology. *Nat Methods*. 2012;9:245–53.
31. Gawish R, Starkl P, Pimenov L, Hladik A, Lakovits K, Oberndorfer F, et al. ACE2 is the critical in vivo receptor for SARS-CoV-2 in a novel COVID-19 mouse model with TNF- and IFN $\gamma$ -driven immunopathology. *eLife*. 2022;11:e74623.
32. Levine KS, Leonard HL, Blauwendraat C, Iwaki H, Johnson N, Bandres-Ciga S, et al. Virus exposure and neurodegenerative disease risk across national biobanks. *Neuron*. 2023;111:1086–1093.e2.
33. Rahmati M, Udeh R, Yon DK, Lee SW, Dolja-Gore X, McEVoy M, et al. A systematic review and meta-analysis of long-term sequelae of COVID-19 2-year after SARS-CoV-2 infection: A call to action for neurological, physical, and psychological sciences. *J Med Virol*. 2023;95:e28852.
34. Rahmati M, Yon DK, Lee SW, Soysal P, Koyanagi A, Jacob L, et al. New-onset neurodegenerative diseases as long-term sequelae of SARS-CoV-2 infection: A systematic review and meta-analysis. *J Med Virol*. 2023;95:e28909.
35. Besteher B, Machnik M, Troll M, Toepffer A, Zerekidze A, Rocktäschel T, et al. Larger gray matter volumes in neuropsychiatric long-COVID syndrome. *Psychiatry Res*. 2022;317:114836.
36. Lu Y, Li X, Geng D, Mei N, Wu P-Y, Huang C-C, et al. Cerebral Micro-Structural Changes in COVID-19 Patients - An MRI-based 3-month Follow-up Study. *EClinicalMedicine*. 2020;25:100484.
37. Kordower JH, Chu Y, Hauser RA, Freeman TB, Olanow CW. Lewy body-like pathology in long-term embryonic nigral transplants in Parkinson's disease. *Nat Med*. 2008;14:504–6.
38. Blanco-Palmero VA, Azcárate-Díaz FJ, Ruiz-Ortiz M, Laespada-García MI, Rábano-Suárez P, Méndez-Guerrero A, et al. Serum and CSF alpha-synuclein levels do not change in COVID-19 patients with neurological symptoms. *J Neurol*. 2021;268:3116–24.
39. Schwabenland M, Salié H, Tanevski J, Killmer S, Lago MS, Schlaak AE, et al. Deep spatial profiling of human COVID-19 brains reveals neuroinflammation with distinct microanatomical microglia-T-cell interactions. *Immunity*. 2021;54:1594–1610.e11.

40. Matschke J, Lütgehetmann M, Hagel C, Sperhake JP, Schröder AS, Edler C, et al. Neuropathology of patients with COVID-19 in Germany: a post-mortem case series. *Lancet Neurol.* 2020;19:919–29.
41. Tsuboi Y, Wszolek ZK, Graff-Radford NR, Cookson N, Dickson DW. Tau pathology in the olfactory bulb correlates with Braak stage, Lewy body pathology and apolipoprotein ε4. *Neuropathol Appl Neurobiol.* 2003;29:503–10.
42. Chiricosta L, Gugliandolo A, Mazzon E. SARS-CoV-2 Exacerbates Beta-Amyloid Neurotoxicity, Inflammation and Oxidative Stress in Alzheimer's Disease Patients. *Int J Mol Sci.* 2021;22:13603.
43. Frontera JA, Boutajangout A, Masurkar AV, Betensky RA, Ge Y, Vedvyas A, et al. Comparison of serum neurodegenerative biomarkers among hospitalized COVID-19 patients versus non-COVID subjects with normal cognition, mild cognitive impairment, or Alzheimer's dementia. *Alzheimers Dement.* 2022;18:899–910.
44. Hsu JT-A, Tien C-F, Yu G-Y, Shen S, Lee Y-H, Hsu P-C, et al. The Effects of Aβ1-42 Binding to the SARS-CoV-2 Spike Protein S1 Subunit and Angiotensin-Converting Enzyme 2. *Int J Mol Sci.* 2021;22:8226.
45. Ramani A, Müller L, Ostermann PN, Gabriel E, Abida-Islam P, Müller-Schiffmann A, et al. SARS-CoV-2 targets neurons of 3D human brain organoids. *EMBO J.* 2020;39:e106230.
46. Eimer WA, Vijaya Kumar DK, Navalpur Shanmugam NK, Rodriguez AS, Mitchell T, Washicosky KJ, et al. Alzheimer's Disease-Associated β-Amyloid Is Rapidly Seeded by Herpesviridae to Protect against Brain Infection. *Neuron.* 2018;99:56-63.e3.
47. Tremblay C, Serrano GE, Intoria AJ, Sue LI, Wilson JR, Adler CH, et al. Effect of olfactory bulb pathology on olfactory function in normal aging. *Brain Pathol.* 2022;n/a:e13075.
48. Balint B, Bhatia KP. Friend or foe? IgLON5 antibodies in a novel tauopathy with prominent sleep movement disorder, ataxia, and chorea. *Mov Disord Off J Mov Disord Soc.* 2014;29:989.
49. Ni Y, Feng Y, Shen D, Chen M, Zhu X, Zhou Q, et al. Anti-IgLON5 antibodies cause progressive behavioral and neuropathological changes in mice. *J Neuroinflammation.* 2022;19:140.
50. Shutinoski B, Hakimi M, Harmsen IE, Lunn M, Rocha J, Lengacher N, et al. Lrrk2 alleles modulate inflammation during microbial infection of mice in a sex-dependent manner. *Sci Transl Med.* 2019;11:eaas9292.

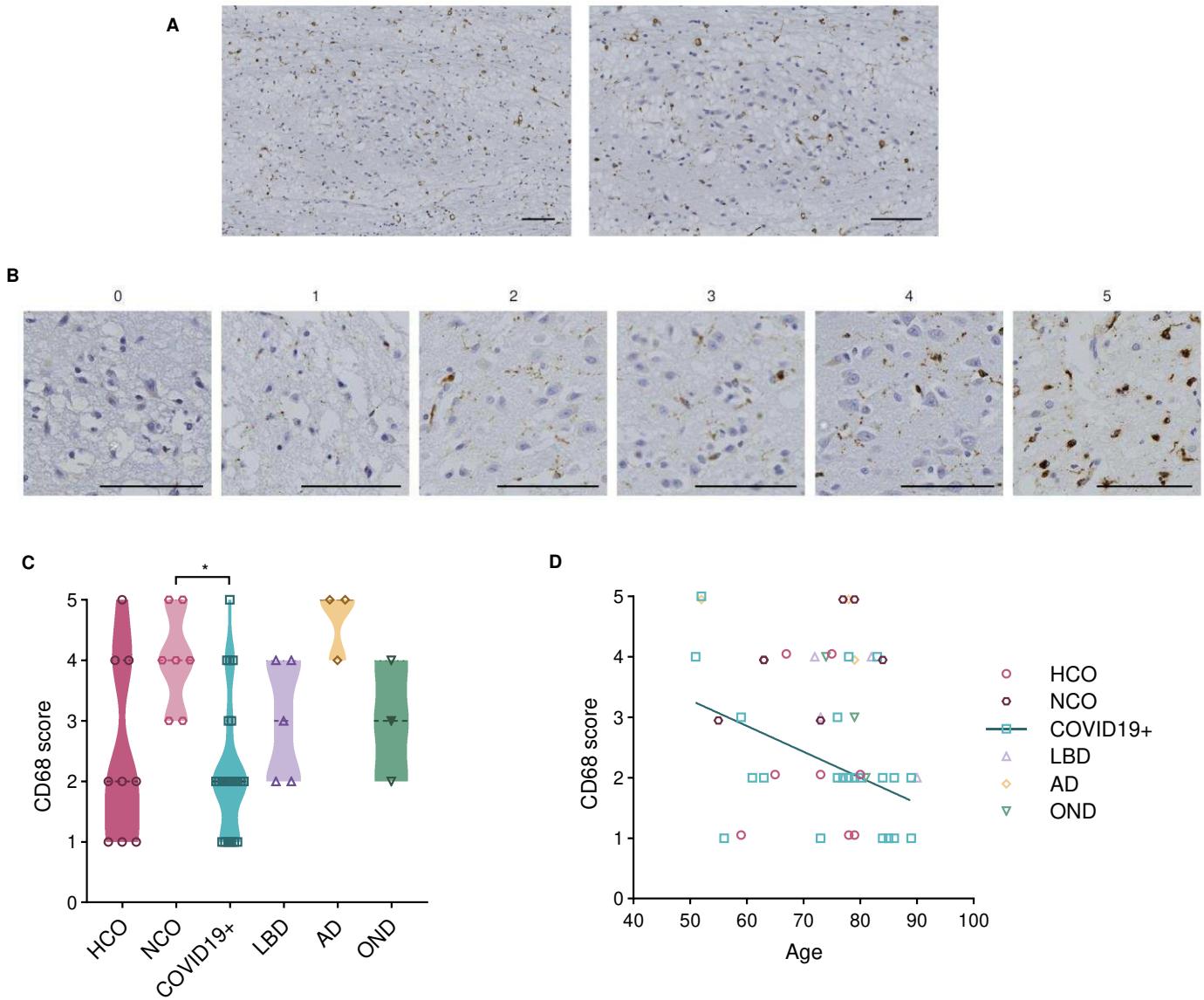
**Table 1:** Demographics of study group.

	N	Age (mean $\pm$ SD)	Age (yrs, range)	Sex		PMI (hrs, mean $\pm$ SD)
				M	F	
<b>Controls</b>	16	70.4 $\pm$ 8.2	55-84	7	9	68.3 $\pm$ 87.0
<b>Healthy Controls</b>	9	72.8 $\pm$ 7.5	59-80	4	5	52.1 $\pm$ 24.3
<b>Neurological Controls</b>	7	70.6 $\pm$ 10.5	55-84	3	4	89.1 $\pm$ 131.2
<b>COVID19+</b>	22	74.8 $\pm$ 12.1	51-89	13	9	43.9 $\pm$ 25.2
<b>Lewy Body Disease</b>	6	78.7 $\pm$ 6.7	72-90	6	0	104.0 $\pm$ 111.2
<b>Alzheimer's Disease</b>	3	69.7 $\pm$ 15.3	52-78	1	2	56.0 $\pm$ 55.4
<b>Other Neurodegenerative Disease</b>	3	78.0 $\pm$ 3.6	74-79	3	0	112.0 $\pm$ 113.4

**A****B****C**

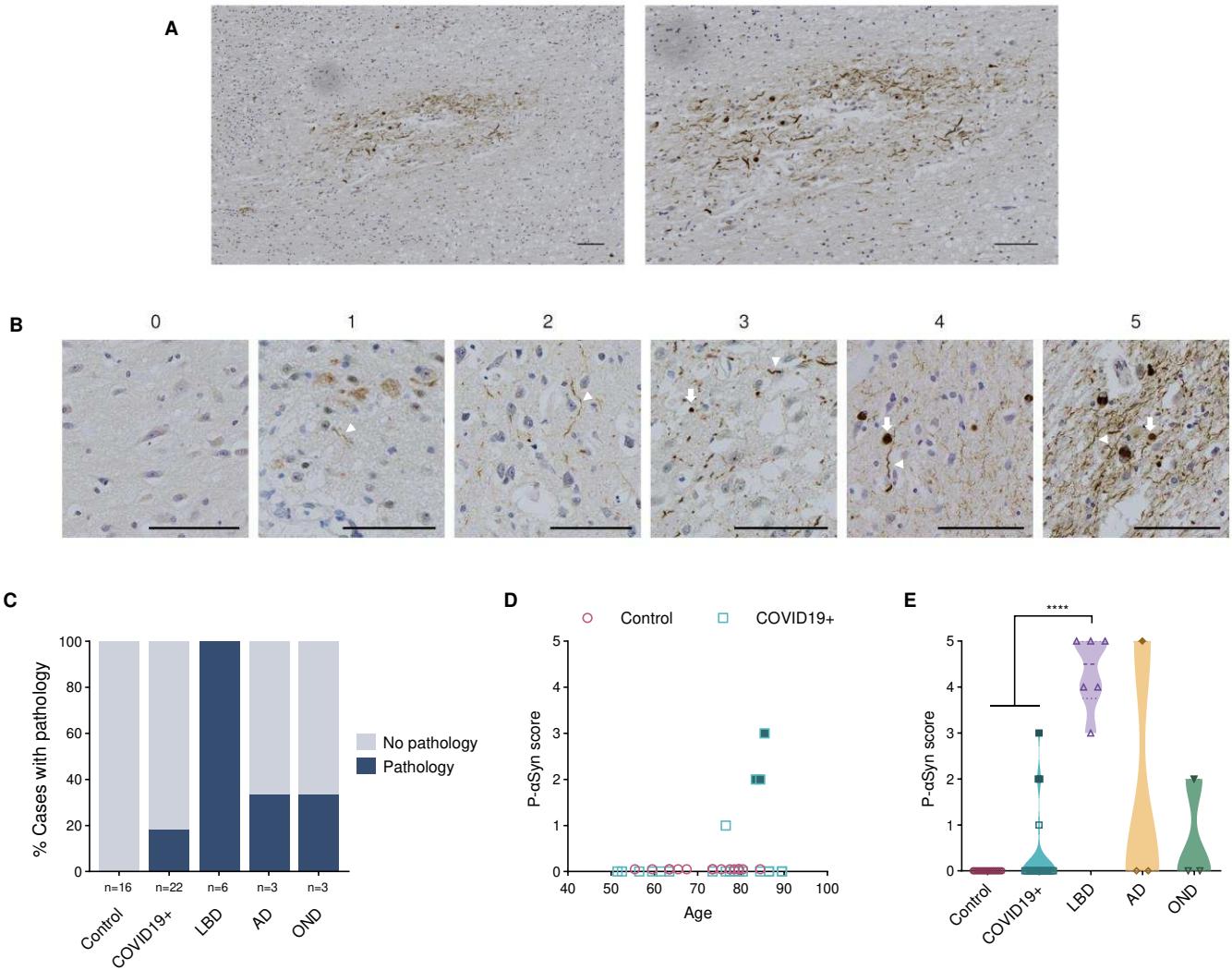
**Figure 1: Overview of anatomical structures in the human olfactory bulb and tract.**

**A)** Hematoxylin and eosin (H&E)-stained section of a human olfactory bulb and tract [case #23] revealing three intrafascicular anterior olfactory nuclei (AON), as outlined in yellow, and in **B)** at higher magnification the most rostrally located AON is shown. **C)** H&E-stained section showing corpora amylacea (in blue) located in the subpial area of the human olfactory tract. Scale bars represent 100 $\mu$ M.



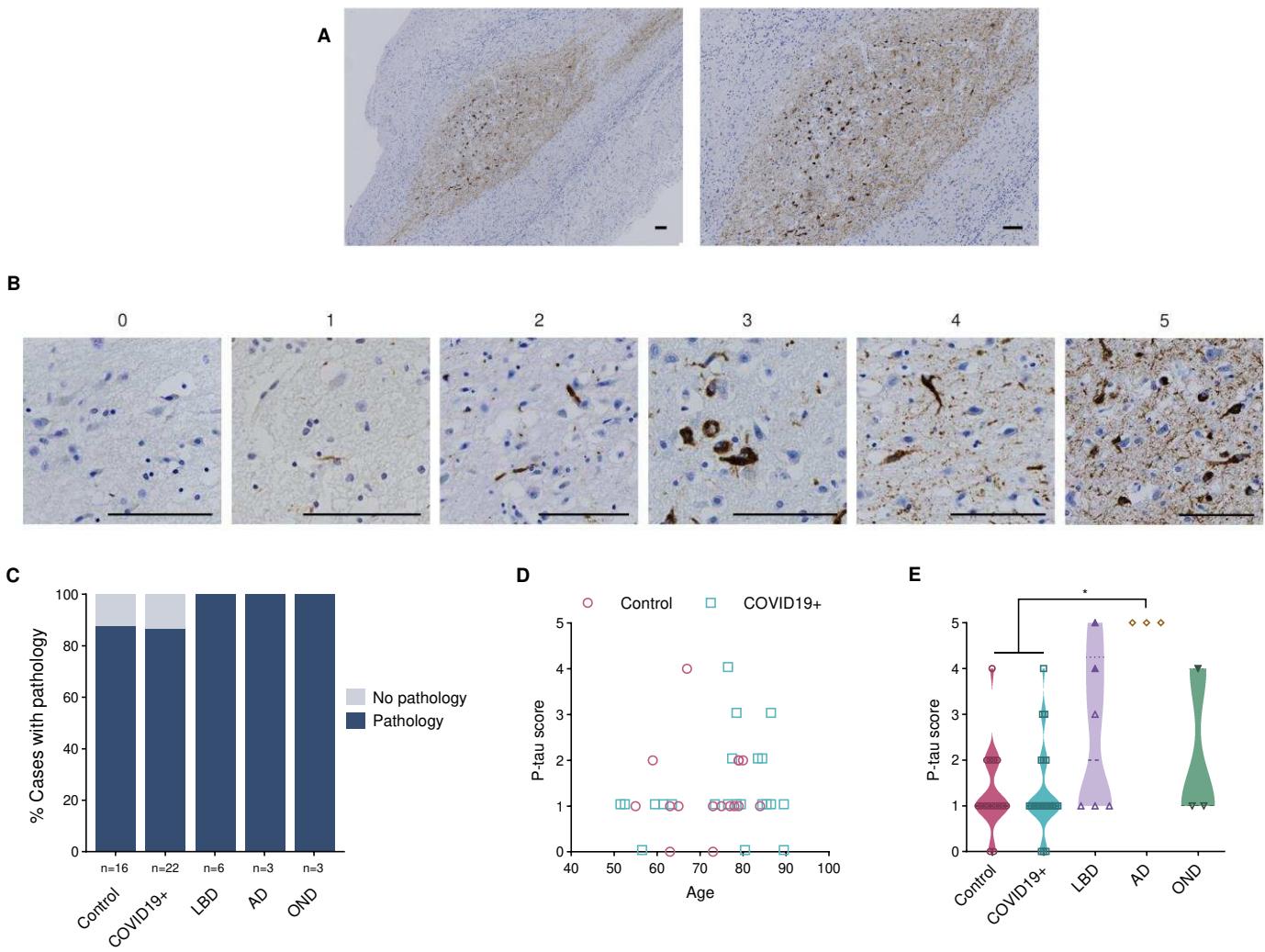
**Figure 2: Anti-CD68 reactivity in the anterior olfactory nucleus.**

**A)** Example of immunohistochemical staining for CD68 in the human olfactory bulb [case #10]. **B)** Representative images of semi-quantitative scoring of staining, scale from 0 to 5, in the AON. **C)** Average staining score for each diagnostic group; filled triangle in the OND group indicates multiple system atrophy case. **D)** Scatter plot showing correlation between age and CD68 score for all groups. Scale bars represent 100 $\mu$ M. Significance was determined using Kruskal-Wallis test with Dunn's post-hoc, where \* indicates  $p\leq 0.05$  (C) and Pearson's correlation where  $r=-0.4494$  (D). Straight line in D denotes correlation for COVID19+ cases. HCO denotes neurologically healthy controls; NCO, neurological controls; LBD, Lewy body disease; AD, Alzheimer disease; OND, other neurodegenerative diseases.



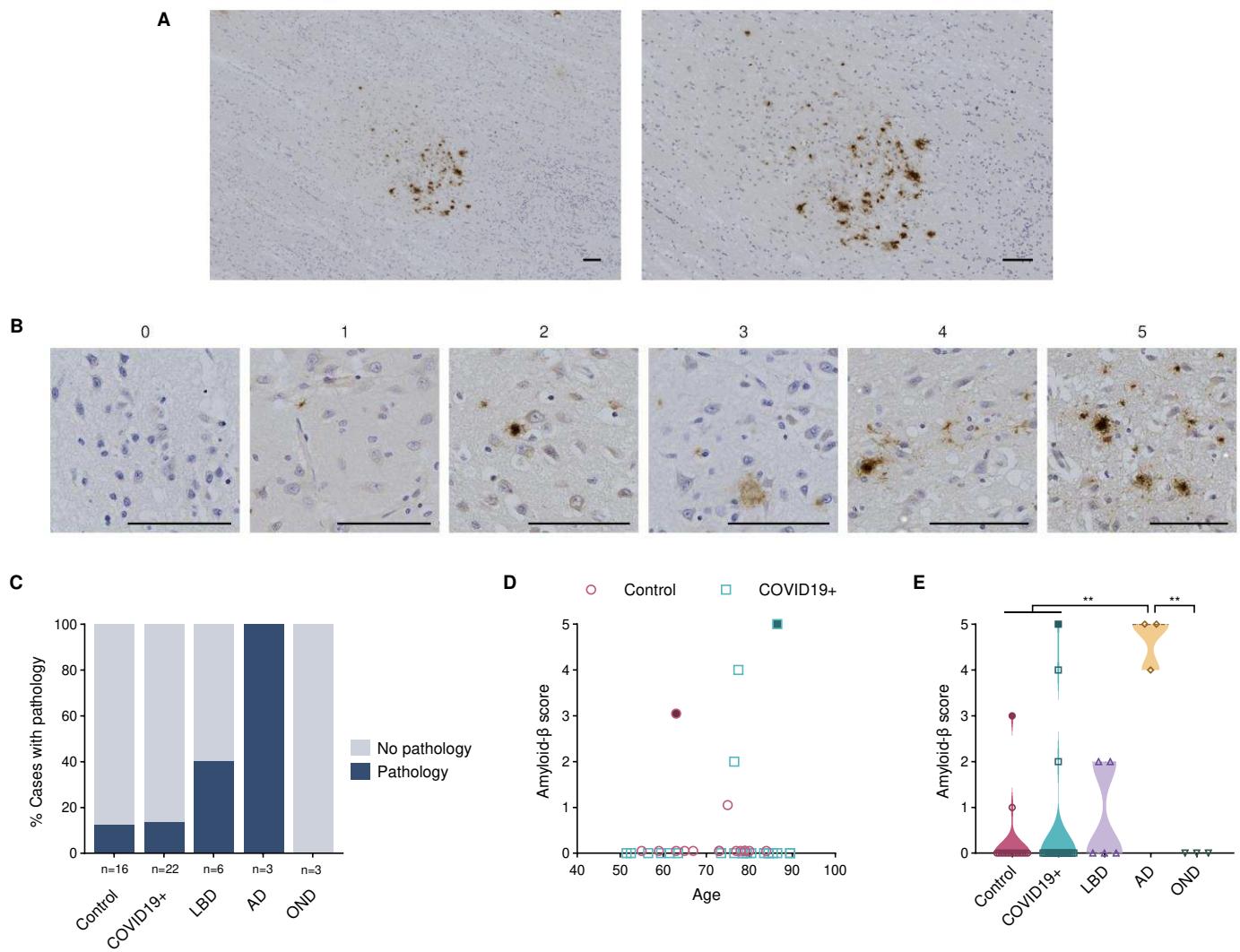
**Figure 3: Anti-phosphorylated  $\alpha$ -synuclein reactivity in the anterior olfactory nucleus.**

**A)** Example of immunohistochemical staining for p- $\alpha$ Syn in the human olfactory bulb, highlighting the AON from a subject with Parkinson disease dementia [case #39]. **B)** Representative images of semi-quantitative scoring of pathology, ranging from 0 to 5, in the AON. White arrows and arrowheads indicate Lewy inclusions and Lewy neurites, respectively. **C)** Percentage of cases in each group that have a pathology score of 1 or more. **D)** Correlation between age and p- $\alpha$ Syn pathology scores in the control group (HCO and NCO) and COVID19+ cases. **E)** Distribution of pathology scores for each group. Filled blue squares in D and E indicate COVID19+ cases suspected of having incidental LBD at autopsy; filled dark yellow diamond indicates AD case diagnosed with mixed pathology at autopsy; filled green triangle indicates MSA case. Scale bars represent 100 $\mu$ M. Significance was determined using Kruskal-Wallis test with Dunn's post-hoc (E), where \*\*\*\* indicates  $p \leq 0.0001$ . Abbreviations for disease groups as in Figure 1.



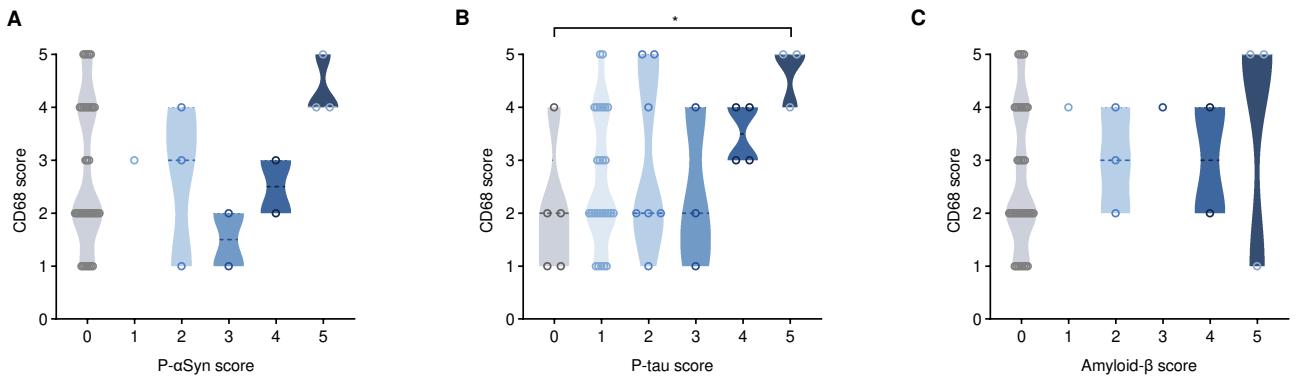
**Figure 4: Anti-phosphorylated tau reactivity in the anterior olfactory nucleus.**

**A)** Example of immunohistochemical staining for p-tau in the human olfactory bulb, concentrated in the AON, from a subject with Alzheimer's disease [case #47]. **B)** Representative images of semi-quantitative scoring of pathology, from 0 to 5, in the AON. **C)** Percentage of cases in each group that have a pathology score of 1 or more. **D)** Correlation between age and p-tau scores for the control (HCO and NCO) and COVID19+ groups. **E)** Distribution of tau pathology score for each group; filled purple triangles indicate LBD cases diagnosed with mixed pathology at autopsy. Filled green triangle indicates MSA case. Scale bar represents 100 $\mu$ M. Significance was determined using Kruskal-Wallis test with Dunn's post-hoc, where \* indicates  $p \leq 0.05$ . Abbreviations for disease groups as in Figure 1.



**Figure 5: Anti-amyloid- $\beta$  peptide reactivity in the anterior olfactory nucleus.**

**A)** Example of immunohistochemical staining for amyloid- $\beta$  peptide in human olfactory bulb, concentrated in the AON, from a subject with Alzheimer's disease [case #46]. **B)** Representative images of semi-quantitative scoring of pathology, from 0 to 5, in the AON. **C)** Percentage of cases in each group that have a pathology score of 1 or more. **D)** Correlation between age and amyloid- $\beta$  peptide scores in the control (HCO and NCO) and COVID19+ groups. Subject highlighted in blue is suspected to have Alzheimer's disease (AD) based on neuropathological findings in the temporal lobes. **E)** Distribution of pathology score for each group. Filled maroon circle indicates a control subject with amyloid- $\beta$ -related angiitis; filled blue square indicates COVID19+ case suspected of having AD. Scale bar represents 100 $\mu$ M. Significance was determined using Kruskal-Wallis test with Dunn's post-hoc, where \*\* indicates  $p \leq 0.01$ . Abbreviations for disease groups as in Figure 1.



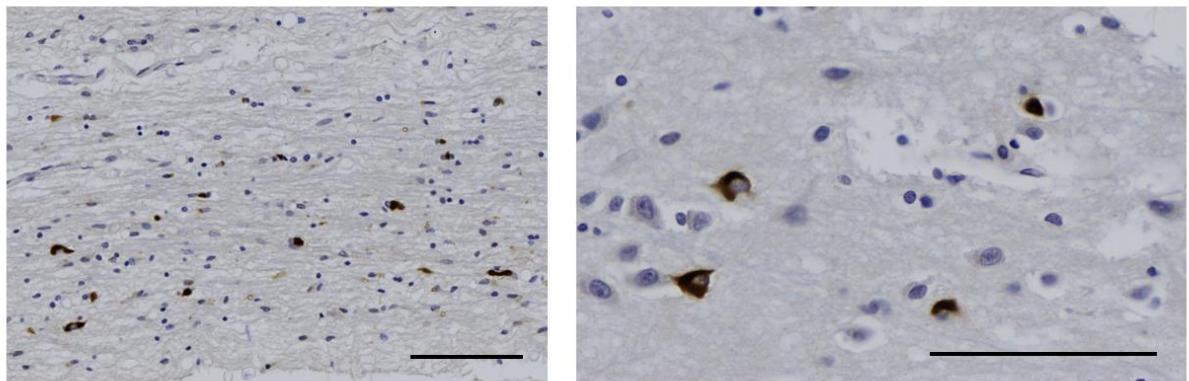
**Figure 6: Correlation studies between inflammation and neurodegeneration-linked pathology in the anterior olfactory nucleus.**

Violin plots showing relationships between scores for anti-CD68 immunoreactivity and three neurodegeneration-linked proteins in the AON, namely phosphorylated  $\alpha$ -synuclein (A), phosphorylated tau (B) and amyloid- $\beta$  peptide (C). Significance was determined using Kruskal-Wallis test with Dunn's post-hoc, using a score of 0 (indicating absent pathology) for comparison, where \* indicates  $p \leq 0.05$ .

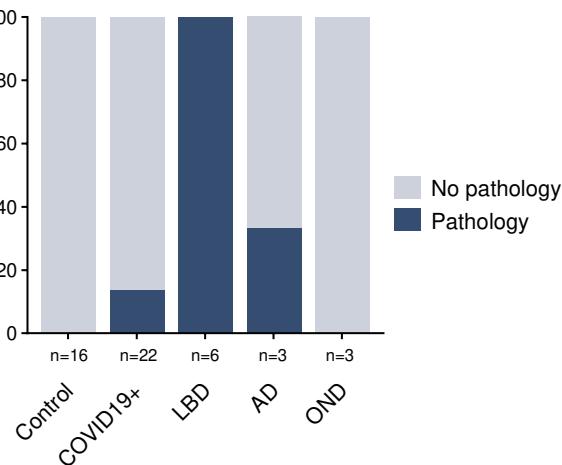
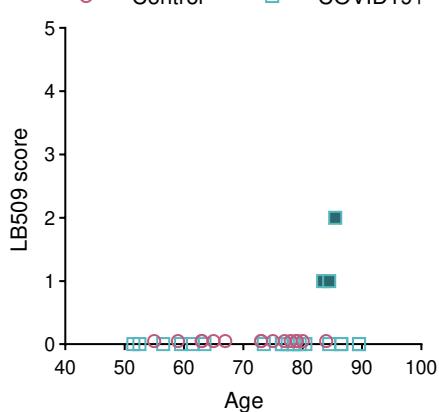
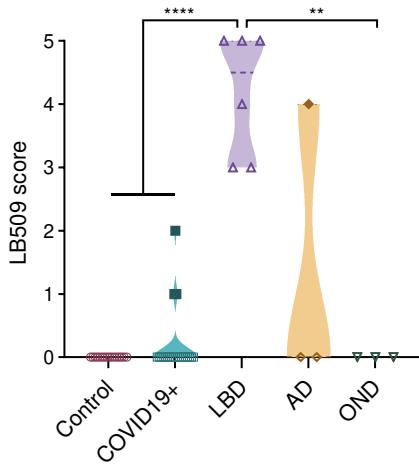
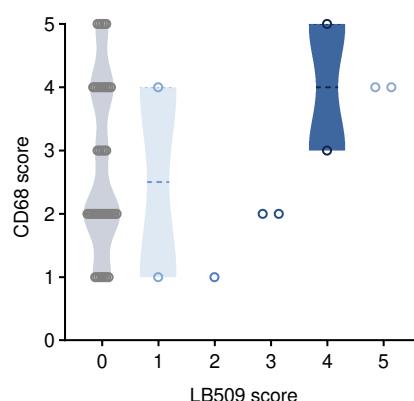
Case ID	Age	Sex	Group	Staining score in AON (from 0-5)			
				P- $\alpha$ Syn	P-tau	Amyloid- $\beta$	CD68
1	55	F	NCO	0	1	0	3
2	59	M	HCO	0	2	0	1
3	63	M	NCO	0	0	0	4
4	63	M	NCO	0	1	3	4
5	65	M	HCO	0	1	0	2
6	67	F	HCO	0	4	0	4
7	73	F	HCO	0	0	0	2
8	73	F	NCO	0	1	0	3
9	75	F	HCO	0	1	1	4
10	77	M	NCO	0	1	0	5
11	78	M	HCO	0	1	0	1
12	79	F	HCO	0	1	0	1
13	79	F	HCO	0	2	0	5
14	79	F	NCO	0	2	0	5
15	80	M	HCO	0	2	0	2
16	84	F	NCO	0	1	0	4
17	51	M	COVID19+	0	1	0	4
18	52	M	COVID19+	0	1	0	5
19	56	M	COVID19+	0	0	0	1
20	59	M	COVID19+	0	1	0	3
21	61	F	COVID19+	0	1	0	2
22	63	F	COVID19+	0	1	0	2
23	73	F	COVID19+	0	1	0	1
24	76	M	COVID19+	1	4	2	3
25	76	F	COVID19+	0	1	0	2
26	77	F	COVID19+	0	2	4	2
27	78	M	COVID19+	0	3	0	2
28	78	F	COVID19+	0	1	0	4
29	79	M	COVID19+	0	1	0	2
30	80	F	COVID19+	0	0	0	2
31	83	F	COVID19+	2	2	0	4
32	84	M	COVID19+	2	1	0	1
33	84	M	COVID19+	0	2	0	2
34	85	M	COVID19+	3	1	0	1
35	86	M	COVID19+	0	3	5	1
36	86	F	COVID19+	0	1	0	2
37	89	M	COVID19+	0	1	0	2
38	89	M	COVID19+	0	0	0	1
39	72	M	LBD	5	4	2	4
40	73	M	LBD	4	1	0	3
41	77	M	LBD	4	1	0	2
42	79	M	LBD	5	5	-	-
43	82	M	LBD	5	3	0	4
44	90	M	LBD	3	1	2	2
45	52	F	AD	5	5	5	5
46	78	F	AD	0	5	5	5
47	79	M	AD	0	5	4	4
48	74	M	OND	0	1	0	4
49	79	M	OND	2	4	0	3
50	81	M	OND	0	1	0	2

**Table 2: Heat map of all staining scores for each case in this study.**

Characteristics listed include age (in years); sex (F, female; M, male); diagnostic group (HCO, healthy control; NCO, neurological control; LBD, Lewy Body Disease' AD, Alzheimer's disease; OND, other neurological disease); and pathology scores in the AON (anterior olfactory nucleus) for each marker, from 0 to 5.



**Supplemental Figure 1:** Example of phosphorylated  $\alpha$ -synuclein pathology shown at low and high magnifications depicting glial cytoplasmic inclusions in oligodendrocytes of the anterior olfactory nucleus in the OB of an individual with multiple system atrophy (type P). Scale bars represent 100 $\mu$ M.

**A****B****C****D**

**Supplemental Figure 2: LB509-mediated  $\alpha$ -synuclein reactivity in the anterior olfactory nucleus.**  
**A)** Percentage of cases in each group that have a pathology score of 1 or more. **B)** Correlation between age and LB509  $\alpha$ -synuclein pathology scores in the control (HCO and NCO) and COVID19+ groups. **C)** Distribution of LB509 pathology scores for each group. Filled blue squares are subjects suspected of having incidental LBD, filled dark yellow diamond reflects a subject with mixed pathology. **D)** Relationship between anti-CD68 score and LB509  $\alpha$ -synuclein pathology score in the AON of all groups. Significance was determined using Kruskal-Wallis test with Dunn's post-hoc, where \*\* denotes  $p \leq 0.01$  and \*\*\*\* denotes  $p \leq 0.0001$ . Abbreviations for disease groups as in Figure 1.

**Supplemental Table 1: Detailed characteristics of study group.** See legend on next page.

Case ID	Age	Sex	PMI (hrs)	Group	Diagnosis at autopsy	Days between admission and death	Site	Tissue	HC staining	aSyn SN/NDV staining
1	55	F	~48	Control*	Adult onset leukoencephalopathy with spheroids and pigmented glia	NA	Ottawa	Olfactory bulb (both) Olfactory tract (both)	NA	N
2	59	M	~72	Control	Normal	NA	Ottawa	Olfactory bulb + tract (both)	NA	N
3	63	M	~24	Control*	CNS inflammatory/demyelinating disorder NOS	NA	Ottawa	Olfactory tract	NA	N
4	63	M	~384	Control*	ABRA (amyloid $\beta$ -related angiitis)	NA	Ottawa	Olfactory bulb + tract (both)	NA	N
5	65	M	13.5	Control	Cardiac hypertrophy; lung edema	2	St. Gallen	Olfactory tract (right) Olfactory tract (left)	A0B1	N
6	67	F	~72	Control	Acute myeloid leukemia	NA	Ottawa	Olfactory tract (both)	NA	N
7	73	F	70	Control	Metastasized breast cancer; cardiac hypertrophy	4	St. Gallen	Olfactory tract (right)	A0B1	N
8	73	F	~24	Control*	Lymphomatosis cerebri	NA	Ottawa	Olfactory bulb (both) Olfactory tract (both)	NA	N
9	75	F	~72	Control	Lung cancer; coronary disease	NA	Ottawa	Olfactory bulb + tract	NA	N
10	77	M	~72	Control*	Meningioma	NA	Ottawa	Olfactory tract	NA	N
11	78	M	22	Control	Metastasized esophageal cancer; mediastinitis/pleuritis; cardiac hypertrophy	9	St. Gallen	Olfactory bulb + tract (right)	A1B2	N
12	79	F	~72	Control	Normal	NA	Ottawa	Olfactory bulb Olfactory tract (both)	NA	N
13	79	F	36	Control	Severe aortic stenosis; cardiac hypertrophy	10	St. Gallen	Olfactory bulb + tract (right) Olfactory bulb + tract (left)	A1B1	N
14	79	F	~48	Control*	Craniocerebral trauma	NA	Ottawa	Olfactory bulb Olfactory bulb + tract	NA	N
15	80	M	39.5	Control	AML infiltration in spleen, liver and kidneys; epicarditis, pericarditis	9	St. Gallen	Olfactory bulb + tract	NA	N
16	84	F	<24	Control*	Acute hemorrhagic leukoencephalopathy	NA	Ottawa	Olfactory bulb + tract (both)	NA	N
17	51	M	23	COVID19+	DAD; bacterial pneumonia	27	St. Gallen	Olfactory bulb + tract (right) Olfactory bulb + tract (left)	A0B1	N
18	52	M	~72	COVID19+	Cerebrovascular disease + Covid-19	NA	Ottawa	Olfactory bulb Olfactory tract	NA	N
19	56	M	33	COVID19+	Pulmonary embolism; chronic myocarditis, cardiac hypertrophy; organizing pneumonia; old lacunes	5	St. Gallen	Olfactory bulb + tract (right) Olfactory bulb + tract (left)	A0B1	N
20	59	M	39	COVID19+	Liver cirrhosis; viral pneumonia; cardiac hypertrophy	49	St. Gallen	Olfactory bulb + tract (right) Olfactory bulb + tract (left)	NA	N
21	61	F	15	COVID19+	Pleural hemorrhage, minimal organizing pneumonia	7	St. Gallen	Olfactory bulb + tract (right) Olfactory bulb + tract (left)	NA	N
22	63	F	24	COVID19+	Pulmonary embolism; bacterial pneumonia; organizing DAD	14	St. Gallen	Olfactory bulb + tract (right) Olfactory tract (left)	A0B1	N
23	73	F	64	COVID19+	Retroperitoneal hemorrhage, pulmonary embolism with lung infarction; cardiac hypertrophy	5	St. Gallen	Olfactory bulb + tract (right) Olfactory bulb + tract (left)	A2B1	N
24	76	M	29	COVID19+	DAD, organizing pneumonia; leg vein thrombosis	11	St. Gallen	Olfactory bulb + tract (right) Olfactory bulb + tract (left)	NA	N
25	76	F	23	COVID19+	Retroperitoneal hemorrhage; DAD, bacterial pneumonia	7	St. Gallen	Olfactory bulb + tract (right) Olfactory bulb + tract (left)	A0B2	N

Case ID	Age	Sex	PMI (hrs)	Group	Diagnosis/cause of death	Days between admission and death	Site	Tissue	HC staining	aSyn SN/DNV staining
26	77	F	87	COVID19+	DAD, bacterial pneumonia; cardiac hypertrophy	13	St. Gallen	Olfactory tract (both)	NA	N
27	78	M	21	COVID19+	Viral pneumonia, microembolisms, DAD; cardiac hypertrophy	6	St. Gallen	Olfactory bulb + tract (both)	A0B2	N
28	78	F	30	COVID19+	Ischemia of the inner cardiac wall, cardiac hypertrophy; old stroke lesions	0	St. Gallen	Olfactory bulb (right) Olfactory bulb (left)	A2B2	N
29	79	M	85	COVID19+	Bacterial pneumonia; old stroke lesion	1	St. Gallen	Olfactory bulb + tract (right) Olfactory bulb + tract (left)	NA	N
30	80	F	71	COVID19+	Organizing pneumonia, acute pneumonia; cardiac hypertrophy	1	St. Gallen	Olfactory bulb + tract (right) Olfactory bulb + tract (left)	A2B1	N
31	83	F	67	COVID19+	DAD, bacterial pneumonia	5	St. Gallen	Olfactory bulb + tract (right) Olfactory bulb + tract (left)	A0B2	Y
32	84	M	24	COVID19+	Pulmonary metastases (colorectal cancer); cardiac hypertrophy	8	St. Gallen	Olfactory bulb + tract (both)	NA	Y
33	84	M	27	COVID19+	Cardiac hypertrophy and fibrosis	3	St. Gallen	Olfactory bulb + tract (right) Olfactory bulb + tract (left)	A0B1	N
34	85	M	66	COVID19+	Retroperitoneal hemorrhage; organizing pneumonia; cardiac hypertrophy. Suspected Parkinsonism.	6	St. Gallen	Olfactory bulb + tract (right) Olfactory bulb + tract (left)	NA	Y
35	86	M	21	COVID19+	DAD, bacterial pneumonia	22	St. Gallen	Olfactory tract (right) Olfactory bulb + tract (left)	A3B2	N
36	86	F	88	COVID19+	DAD, bacterial pneumonia, little thrombi in peripheral vessels in lung; cardiac hypertrophy	7	St. Gallen	Olfactory bulb + tract (right) Olfactory bulb + tract (left)	A0B1	N
37	89	M	33	COVID19+	Multiple myocardial infarctions, cardiac hypertrophy	12	St. Gallen	Olfactory bulb + tract (right) Olfactory bulb + tract (left)	A0B2	N
38	89	M	~24	COVID19+	Cerebrovascular disease + Covid	NA	Ottawa	Olfactory bulb + tract (both)	NA	N
39	72	M	~24	LBD <sup>#</sup>	DLB and A3B2C1	NA	Ottawa	Olfactory bulb + tract	NA	Y
40	73	M	~144	LBD	DLB and A1B1C1	NA	Ottawa	Olfactory tract Olfactory bulb	NA	Y
41	77	M	~24	LBD	LBD early limbic, FTLD-TDP A, possible ALS	NA	Ottawa	Olfactory bulb	NA	N
42	79	M	~48	LBD <sup>#</sup>	LBD diffuse neocortical stage, and AD-related changes (A3B1C0)	NA	Ottawa	Olfactory bulb + tract	NA	Y
43	82	M	~72	LBD	DLB, neocortical stage	NA	Ottawa	Olfactory bulb + tract Olfactory bulb	NA	Y
44	90	M	~312	LBD	DLB early neocortical stage	NA	Ottawa	Olfactory tract	NA	Y
45	52	F	~120	AD <sup>#</sup>	AD (PSEN1) + DLB early cortical	NA	Ottawa	Olfactory bulb + tract (both)	NA	Y
46	78	F	<24	AD	AD (A3B3C3)	NA	Ottawa	Olfactory bulb + tract Olfactory bulb + tract	NA	N
47	79	M	~24	AD	AD (A3B3C3)	NA	Ottawa	Olfactory bulb + tract Olfactory bulb + tract	NA	N
48	74	M	~72	OND	PSP	NA	Ottawa	Olfactory bulb + tract	NA	N
49	79	M	<24	OND	MSA	NA	Ottawa	Olfactory tract (both)	NA	Y
50	81	M	~240	OND	PSP	NA	Ottawa	Olfactory tract	NA	N

### Supplemental Table 1: Detailed characteristics of study group.

Characteristics listed include age (in years); sex (F, female; M, male); *post mortem* interval (PMI; in hours); diagnostic group (HCO, healthy control; NCO, neurological control; LBD, Lewy Body Disease' AD, Alzheimer's disease; OND, other neurological disease); cause of death or diagnosis at autopsy; number of days in hospital, NA, not applicable; site of tissue collection; tissue analyzed; hippocampal (HC) staining; aSynuclein staining in the substantia nigra (SN) and dorsal motor nucleus of the vagus (DNV).\*Indicate cases with an inflammatory condition. #Indicate cases with mixed pathology.

**Supplemental Table 2:** Resources and reagents used in study

Reagent or Resource	Source	Identifier
<b>Antibodies</b>		
Anti-phosphorylated $\alpha$ Synuclein	FUJIFILM Wako Shibayagi	Cat# 015-25191 RRID:AB_2537218
Phospho-Tau (Ser202, Thr205) Monoclonal Antibody (AT8)	Thermo Fisher Scientific	Cat# MN1020 RRID:AB_223647
KiM1P	Non-commercial. Gift from J. Franz	As described by Radzun et al. 1991
Purified (azide-free) anti-beta-Amyloid, 1-16	Biolegend	Cat# 803001 RRID:AB_2564653
LB509 [Purified (azide-free) anti-alpha-Synuclein, 115-121]	Biolegend	Cat# 807702 RRID:AB_2564725
SARS Nucleocapsid protein	Novus Biologicals	Cat# NB100-56576 RRID: AB_838838
Anti-Mouse IgG (H+L), made in goat	Vector Laboratories	Cat# BA-9200 RRID:AB_2336171
Anti-Rabbit IgG (H+L), made in goat	Vector Laboratories	Cat# BA-1000 RRID:AB_2313606
<b>Reagent</b>		
VECTASTAIN Elite ABC-Peroxidase Kit	Vector Laboratories	Cat# PK-6100 RRID:AB_2336819
SIGMAFAST™ 3,3'-Diaminobenzidine tablets	Millipore Sigma	Cat# D4293
Permount Mounting Medium	Fisher Scientific	Cat# SP15-100

**Supplemental Table 3: Phosphorylated  $\alpha$ -Synuclein staining.**

Case ID	Age	Sex	Group	Tissue	# of AON	AON score	SN/DNV pathology
1	55	F	Control	Olfactory bulb (both)	1	0	N
				Olfactory tract (both)	1	0	
2	59	M	Control	Olfactory bulb + tract (both)	3	0	N
3	63	M	Control	Olfactory tract	1	0	N
4	63	M	Control	Olfactory bulb + tract (both)	4	0	N
5	65	M	Control	Olfactory tract (right)	2	0	N
				Olfactory tract (left)	1	0	
6	67	F	Control	Olfactory tract (both)	4	0	N
7	73	F	Control	Olfactory tract (right)	2	0	N
8	73	F	Control	Olfactory bulb (both)	2	0	N
				Olfactory tract (both)	1	0	
9	75	F	Control	Olfactory bulb + tract	3	0	N
10	77	M	Control	Olfactory tract	3	0	N
11	78	M	Control	Olfactory bulb + tract (right)	3	0	N
				Olfactory bulb	4	0	
				Olfactory tract (both)	3	0	
13	79	F	Control	Olfactory bulb + tract (right)	2	0	N
				Olfactory bulb + tract (left)	1	0	
14	79	F	Control	Olfactory bulb	1	0	N
				Olfactory bulb + tract	4	0	
15	80	M	Control	Olfactory bulb + tract	3	0	N
16	84	F	Control	Olfactory bulb + tract (both)	5	0	N
17	51	M	COVID19+	Olfactory bulb + tract (right)	2	0	N
				Olfactory bulb + tract (left)	5	0	
18	52	M	COVID19+	Olfactory bulb	3	0	N
				Olfactory tract	4	0	
19	56	M	COVID19+	Olfactory bulb + tract (right)	1	0	N
				Olfactory bulb + tract (left)	0	0	
20	59	M	COVID19+	Olfactory bulb + tract (right)	2	0	N
				Olfactory bulb + tract (left)	5	0	
21	61	F	COVID19+	Olfactory bulb + tract (right)	2	0	N
				Olfactory bulb + tract (left)	1	0	
22	63	F	COVID19+	Olfactory bulb + tract (right)	2	0	N
				Olfactory tract (left)	1	0	
23	73	F	COVID19+	Olfactory bulb + tract (right)	1	0	N
				Olfactory bulb + tract (left)	1	0	
24	76	M	COVID19+	Olfactory bulb + tract (right)	5	1	N
				Olfactory bulb + tract (left)	2	0	
25	76	F	COVID19+	Olfactory bulb + tract (right)	3	0	N
				Olfactory bulb + tract (left)	3	0	

**Supplemental Table 3 cont'd**

Case ID	Age	Sex	Group	Tissue	# of AON	AON score	SN/DNV pathology
26	77	F	COVID19+	Olfactory tract (both)	1	0	N
27	78	M	COVID19+	Olfactory bulb + tract (both)	1	0	N
28	78	F	COVID19+	Olfactory bulb (right)	2	0	
				Olfactory bulb (left)	2	0	N
29	79	M	COVID19+	Olfactory bulb + tract (right)	4	0	
				Olfactory bulb + tract (left)	2	0	N
30	80	F	COVID19+	Olfactory bulb + tract (right)	3	0	
				Olfactory bulb + tract (left)	2	0	N
31	83	F	COVID19+	Olfactory bulb + tract (right)	2	2	
				Olfactory bulb + tract (left)	3	2	Y
32	84	M	COVID19+	Olfactory bulb + tract (both)	4	2	Y
33	84	M	COVID19+	Olfactory bulb + tract (right)	2	0	
				Olfactory bulb + tract (left)	2	0	N
34	85	M	COVID19+	Olfactory bulb + tract (right)	2	3	
				Olfactory bulb + tract (left)	3	3	Y
35	86	M	COVID19+	Olfactory tract (right)	2	0	
				Olfactory bulb + tract (left)	1	0	N
36	86	F	COVID19+	Olfactory bulb + tract (right)	2	0	
				Olfactory bulb + tract (left)	2	0	N
37	89	M	COVID19+	Olfactory bulb + tract (right)	3	0	
				Olfactory bulb + tract (left)	2	0	N
38	89	M	COVID19+	Olfactory bulb + tract (both)	6	0	N
39	72	M	LBD	Olfactory bulb + tract	5	5	Y
40	73	M	LBD	Olfactory tract	0	0	
				Olfactory bulb	3	4	Y
41	77	M	LBD	Olfactory bulb	4	4	N
42	79	M	LBD	Olfactory bulb + tract	4	5	Y
43	82	M	LBD	Olfactory bulb + tract	2	5	
				Olfactory bulb	2	5	Y
44	90	M	LBD	Olfactory tract	3	3	Y
45	52	F	AD	Olfactory bulb + tract (both)	7	5	Y
46	78	F	AD	Olfactory bulb + tract	3	0	
				Olfactory bulb + tract	3	0	N
47	79	M	AD	Olfactory bulb + tract	2	0	
				Olfactory bulb + tract	3	0	N
48	74	M	OND	Olfactory bulb + tract	1	0	N
49	79	M	OND	Olfactory tract (both)	2	2	Y
50	81	M	OND	Olfactory tract	1	0	N

AON, anterior olfactory nucleus; SN, substantia nigra; DNV, dorsal motor nucleus of the vagus; LBD, Lewy Body disease; AD, Alzheimer's disease; OND, other neurodegenerative disease

**Supplemental Table 4: Phosphorylated tau staining**

Case ID	Age	Sex	Group	Tissue	# of AON	AON score
1	55	F	Control	Olfactory bulb (both)	1	1
				Olfactory tract (both)	2	0
2	59	M	Control	Olfactory bulb + tract (both)	3	2
3	63	M	Control	Olfactory tract	1	0
4	63	M	Control	Olfactory bulb + tract (both)	3	1
				Olfactory tract (right)	1	1
5	65	M	Control	Olfactory tract (left)	2	0
				Olfactory tract (both)	7	4
6	67	F	Control	Olfactory tract (right)	2	0
7	73	F	Control	Olfactory tract (both)	2	0
8	73	F	Control	Olfactory bulb (both)	2	0
				Olfactory tract (both)	1	1
9	75	F	Control	Olfactory bulb + tract	2	1
10	77	M	Control	Olfactory tract	2	1
11	78	M	Control	Olfactory bulb + tract (right)	4	1
				Olfactory bulb	3	2
12	79	F	Control	Olfactory tract (both)	3	2
				Olfactory bulb + tract (right)	2	0
13	79	F	Control	Olfactory bulb + tract (left)	4	1
				Olfactory bulb	1	0
14	79	F	Control	Olfactory bulb + tract	4	2
				Olfactory bulb + tract	3	2
15	80	M	Control	Olfactory bulb + tract	1	1
16	84	F	Control	Olfactory bulb + tract (both)	5	1
17	51	M	COVID19+	Olfactory bulb + tract (right)	3	1
				Olfactory bulb + tract (left)	5	1
18	52	M	COVID19+	Olfactory bulb	5	1
				Olfactory tract	3	1
19	56	M	COVID19+	Olfactory bulb + tract (right)	1	0
				Olfactory bulb + tract (left)	0	0
20	59	M	COVID19+	Olfactory bulb + tract (right)	1	1
				Olfactory bulb + tract (left)	3	1
21	61	F	COVID19+	Olfactory bulb + tract (right)	3	1
				Olfactory bulb + tract (left)	1	0
22	63	F	COVID19+	Olfactory bulb + tract (right)	2	1
				Olfactory tract (left)	1	0
23	73	F	COVID19+	Olfactory bulb + tract (right)	1	0
				Olfactory bulb + tract (left)	2	1
24	76	M	COVID19+	Olfactory bulb + tract (right)	8	4
				Olfactory bulb + tract (left)	8	4
25	76	F	COVID19+	Olfactory bulb + tract (right)	3	1
				Olfactory bulb + tract (left)	3	1

**Supplemental Table 4 cont'd**

Case ID	Age	Sex	Group	Tissue	# of AON	AON score
26	77	F	COVID19+	Olfactory tract (both)	4	2
27	78	M	COVID19+	Olfactory bulb + tract (both)	5	3
28	78	F	COVID19+	Olfactory bulb (right)	1	1
				Olfactory bulb (left)	1	1
29	79	M	COVID19+	Olfactory bulb + tract (right)	3	1
				Olfactory bulb + tract (left)	1	0
30	80	F	COVID19+	Olfactory bulb + tract (right)	3	0
				Olfactory bulb + tract (left)	2	0
31	83	F	COVID19+	Olfactory bulb + tract (right)	4	2
				Olfactory bulb + tract (left)	2	2
32	84	M	COVID19+	Olfactory bulb + tract (both)	3	1
33	84	M	COVID19+	Olfactory bulb + tract (right)	3	2
				Olfactory bulb + tract (left)	1	1
34	85	M	COVID19+	Olfactory bulb + tract (right)	2	1
				Olfactory bulb + tract (left)	1	0
35	86	M	COVID19+	Olfactory tract (right)	3	2
				Olfactory bulb + tract (left)	3	3
36	86	F	COVID19+	Olfactory bulb + tract (right)	2	1
				Olfactory bulb + tract (left)	3	1
37	89	M	COVID19+	Olfactory bulb + tract (right)	3	1
				Olfactory bulb + tract (left)	3	1
38	89	M	COVID19+	Olfactory bulb + tract (both)	7	0
39	72	M	LBD	Olfactory bulb + tract	3	4
40	73	M	LBD	Olfactory tract	0	0
				Olfactory bulb	6	1
41	77	M	LBD	Olfactory bulb	2	1
42	79	M	LBD	Olfactory bulb + tract	4	5
43	82	M	LBD	Olfactory bulb + tract	0	0
				Olfactory bulb	3	3
44	90	M	LBD	Olfactory tract	2	1
45	52	F	AD	Olfactory bulb + tract (both)	5	5
46	78	F	AD	Olfactory bulb + tract	2	5
				Olfactory bulb + tract	3	5
47	79	M	AD	Olfactory bulb + tract	5	5
				Olfactory bulb + tract	5	5
48	74	M	OND	Olfactory bulb + tract	2	1
49	79	M	OND	Olfactory tract (both)	7	4
50	81	M	OND	Olfactory tract	1	1

AON, anterior olfactory nucleus; LBD, Lewy Body disease; AD, Alzheimer's disease; OND, other neurodegenerative disease

**Supplemental Table 5:** Amyloid- $\beta$  staining

Case ID	Age	Sex	Group	Tissue	# of AON	AON score	HC staining
1	55	F	Control	Olfactory bulb (both)	1	0	NA
				Olfactory tract (both)	1	0	
2	59	M	Control	Olfactory bulb + tract (both)	5	0	NA
3	63	M	Control	Olfactory tract	1	0	NA
4	63	M	Control	Olfactory bulb + tract (both)	3	3	NA
5	65	M	Control	Olfactory tract (right)	1	0	A0B1
				Olfactory tract (left)	2	0	
6	67	F	Control	Olfactory tract (both)	4	0	NA
7	73	F	Control	Olfactory tract (right)	1	0	A0B1
8	73	F	Control	Olfactory bulb (both)	2	0	NA
				Olfactory tract (both)	2	0	
9	75	F	Control	Olfactory bulb + tract	2	1	NA
10	77	M	Control	Olfactory tract	2	0	NA
11	78	M	Control	Olfactory bulb + tract (right)	3	0	A1B2
				Olfactory bulb	4	0	
				Olfactory tract (both)	3	0	NA
13	79	F	Control	Olfactory bulb + tract (right)	4	0	A1B1
				Olfactory bulb + tract (left)	2	0	
14	79	F	Control	Olfactory bulb	1	0	NA
				Olfactory bulb + tract	3	0	
15	80	M	Control	Olfactory bulb + tract	2	0	NA
16	84	F	Control	Olfactory bulb + tract (both)	4	0	NA
17	51	M	COVID19+	Olfactory bulb + tract (right)	1	0	A0B1
				Olfactory bulb + tract (left)	3	0	
18	52	M	COVID19+	Olfactory bulb	3	0	NA
				Olfactory tract	2	0	
19	56	M	COVID19+	Olfactory bulb + tract (right)	1	0	A0B1
				Olfactory bulb + tract (left)	0	0	
20	59	M	COVID19+	Olfactory bulb + tract (right)	1	0	NA
				Olfactory bulb + tract (left)	5	0	
21	61	F	COVID19+	Olfactory bulb + tract (right)	5	0	NA
				Olfactory bulb + tract (left)	2	0	
22	63	F	COVID19+	Olfactory bulb + tract (right)	6	0	A0B1
				Olfactory tract (left)	2	0	
23	73	F	COVID19+	Olfactory bulb + tract (right)	1	0	A2B1
				Olfactory bulb + tract (left)	3	0	
24	76	M	COVID19+	Olfactory bulb + tract (right)	4	0	NA
				Olfactory bulb + tract (left)	4	2	
25	76	F	COVID19+	Olfactory bulb + tract (right)	2	0	A0B2
				Olfactory bulb + tract (left)	3	0	

**Supplemental Table 5 cont'd**

Case ID	Age	Sex	Group	Tissue	# of AON	AON score	HC staining
26	77	F	COVID19+	Olfactory tract (both)	4	4	NA
27	78	M	COVID19+	Olfactory bulb + tract (both)	5	0	A0B2
28	78	F	COVID19+	Olfactory bulb (right)	1	0	A2B2
				Olfactory bulb (left)	1	0	
29	79	M	COVID19+	Olfactory bulb + tract (right)	2	0	NA
				Olfactory bulb + tract (left)	2	0	
30	80	F	COVID19+	Olfactory bulb + tract (right)	4	0	A2B1
				Olfactory bulb + tract (left)	2	0	
31	83	F	COVID19+	Olfactory bulb + tract (right)	1	0	A0B2
				Olfactory bulb + tract (left)	2	0	
32	84	M	COVID19+	Olfactory bulb + tract (both)	8	0	NA
33	84	M	COVID19+	Olfactory bulb + tract (right)	1	0	A0B1
				Olfactory bulb + tract (left)	2	0	
34	85	M	COVID19+	Olfactory bulb + tract (right)	1	0	NA
				Olfactory bulb + tract (left)	2	0	
35	86	M	COVID19+	Olfactory tract (right)	2	0	A3B2
				Olfactory bulb + tract (left)	3	5	
36	86	F	COVID19+	Olfactory bulb + tract (right)	2	0	A0B1
				Olfactory bulb + tract (left)	2	0	
37	89	M	COVID19+	Olfactory bulb + tract (right)	3	0	A0B2
				Olfactory bulb + tract (left)	3	0	
38	89	M	COVID19+	Olfactory bulb + tract (both)	7	0	NA
39	72	M	LBD	Olfactory bulb + tract	2	2	NA
40	73	M	LBD	Olfactory tract	0	0	NA
				Olfactory bulb	3	0	
41	77	M	LBD	Olfactory bulb	5	0	NA
42	79	M	LBD	Olfactory bulb + tract	-	-	NA
43	82	M	LBD	Olfactory bulb + tract	0	0	NA
				Olfactory bulb	3	0	
44	90	M	LBD	Olfactory tract	2	2	NA
45	52	F	AD	Olfactory bulb + tract (both)	3	5	NA
46	78	F	AD	Olfactory bulb + tract	3	5	NA
				Olfactory bulb + tract	3	4	
47	79	M	AD	Olfactory bulb + tract	4	4	NA
				Olfactory bulb + tract	1	4	
48	74	M	OND	Olfactory bulb + tract	1	0	NA
49	79	M	OND	Olfactory tract (both)	4	0	NA
50	81	M	OND	Olfactory tract	1	0	NA

AON, anterior olfactory nucleus; HC, hippocampus; LBD, Lewy Body disease; AD, Alzheimer's disease; OND, other neurodegenerative disease

**Supplemental Table 6: CD68 staining**

Case ID	Age	Sex	Group	Tissue	# of AON	AON score
1	55	F	Control	Olfactory bulb (both)	3	3
				Olfactory tract (both)	2	2
2	59	M	Control	Olfactory bulb + tract (both)	4	1
				Olfactory tract	1	4
3	63	M	Control	Olfactory bulb + tract (both)	2	4
				Olfactory tract (right)	1	2
4	63	M	Control	Olfactory tract (left)	1	1
				Olfactory tract (both)	3	4
5	65	M	Control	Olfactory tract (right)	1	2
				Olfactory tract (left)	1	1
6	67	F	Control	Olfactory tract (both)	2	3
7	73	F	Control	Olfactory tract (right)	1	2
				Olfactory bulb (both)	2	3
8	73	F	Control	Olfactory tract (both)	2	3
				Olfactory bulb + tract	1	4
9	75	F	Control	Olfactory tract	4	5
				Olfactory bulb + tract (right)	1	1
10	77	M	Control	Olfactory bulb	3	5
				Olfactory tract (both)	3	5
11	78	M	Control	Olfactory bulb + tract (right)	2	0
				Olfactory bulb + tract (left)	2	1
12	79	F	Control	Olfactory bulb	1	5
				Olfactory bulb + tract	3	5
13	79	F	Control	Olfactory bulb + tract	2	2
				Olfactory bulb + tract (right)	1	3
14	79	F	Control	Olfactory bulb + tract (left)	4	4
				Olfactory bulb	2	5
15	80	M	Control	Olfactory bulb	4	5
				Olfactory bulb + tract	2	2
16	84	F	Control	Olfactory bulb + tract (both)	7	4
				Olfactory bulb + tract (right)	1	3
17	51	M	COVID19+	Olfactory bulb + tract (left)	4	4
				Olfactory bulb	2	5
18	52	M	COVID19+	Olfactory tract	4	4
				Olfactory bulb + tract (right)	1	1
19	56	M	COVID19+	Olfactory bulb + tract (left)	1	1
				Olfactory bulb + tract (right)	1	2
20	59	M	COVID19+	Olfactory bulb + tract (left)	3	3
				Olfactory bulb + tract (right)	5	2
21	61	F	COVID19+	Olfactory bulb + tract (left)	2	2
				Olfactory bulb + tract (right)	5	1
22	63	F	COVID19+	Olfactory tract (left)	2	2
				Olfactory bulb + tract (left)	2	1
23	73	F	COVID19+	Olfactory bulb + tract (right)	2	1
				Olfactory bulb + tract (left)	2	1
24	76	M	COVID19+	Olfactory bulb + tract (right)	5	2
				Olfactory bulb + tract (left)	4	3
25	76	F	COVID19+	Olfactory bulb + tract (right)	2	2
				Olfactory bulb + tract (left)	3	2

**Supplemental Table 6 cont'd**

Case ID	Age	Sex	Group	Tissue	# of AON	AON score
26	77	F	COVID19+	Olfactory tract (both)	2	2
27	78	M	COVID19+	Olfactory bulb + tract (both)	4	2
28	78	F	COVID19+	Olfactory bulb (right)	3	5
				Olfactory bulb (left)	3	5
29	79	M	COVID19+	Olfactory bulb + tract (right)	3	2
				Olfactory bulb + tract (left)	3	1
30	80	F	COVID19+	Olfactory bulb + tract (right)	3	1
				Olfactory bulb + tract (left)	1	2
31	83	F	COVID19+	Olfactory bulb + tract (right)	1	4
				Olfactory bulb + tract (left)	4	4
32	84	M	COVID19+	Olfactory bulb + tract (both)	4	1
33	84	M	COVID19+	Olfactory bulb + tract (right)	4	2
				Olfactory bulb + tract (left)	2	2
34	85	M	COVID19+	Olfactory bulb + tract (right)	2	1
				Olfactory bulb + tract (left)	3	0
35	86	M	COVID19+	Olfactory tract (right)	3	1
				Olfactory bulb + tract (left)	2	1
36	86	F	COVID19+	Olfactory bulb + tract (right)	2	2
				Olfactory bulb + tract (left)	2	2
37	89	M	COVID19+	Olfactory bulb + tract (right)	3	2
				Olfactory bulb + tract (left)	5	2
38	89	M	COVID19+	Olfactory bulb + tract (both)	3	2
39	72	M	LBD	Olfactory bulb + tract	4	4
40	73	M	LBD	Olfactory tract	1	3
				Olfactory bulb	4	3
41	77	M	LBD	Olfactory bulb	4	2
42	79	M	LBD	Olfactory bulb + tract	-	-
43	82	M	LBD	Olfactory bulb + tract	2	4
				Olfactory bulb	2	4
44	90	M	LBD	Olfactory tract	2	2
45	52	F	AD	Olfactory bulb + tract (both)	3	5
46	78	F	AD	Olfactory bulb + tract	3	5
				Olfactory bulb + tract	4	4
47	79	M	AD	Olfactory bulb + tract	2	4
				Olfactory bulb + tract	3	3
48	74	M	OND	Olfactory bulb + tract	3	4
49	79	M	OND	Olfactory tract (both)	4	3
50	81	M	OND	Olfactory tract	1	2

AON, anterior olfactory nucleus; LBD, Lewy Body disease; AD, Alzheimer's disease; OND, other neurodegenerative disease

**Supplemental Table 7: LB509 staining**

Case ID	Age	Sex	Group	Tissue	# of AON	AON score
1	55	F	Control	Olfactory bulb (both)	3	0
				Olfactory tract (both)	3	0
2	59	M	Control	Olfactory bulb + tract (both)	6	0
3	63	M	Control	Olfactory tract	2	0
4	63	M	Control	Olfactory bulb + tract (both)	3	0
5	65	M	Control	Olfactory tract (right)	4	0
				Olfactory tract (left)	4	0
6	67	F	Control	Olfactory tract (both)	8	0
7	73	F	Control	Olfactory tract (right)	2	0
8	73	F	Control	Olfactory bulb (both)	2	0
				Olfactory tract (both)	2	0
9	75	F	Control	Olfactory bulb + tract	2	0
10	77	M	Control	Olfactory tract	2	0
11	78	M	Control	Olfactory bulb + tract (right)	1	0
				Olfactory bulb	2	0
12	79	F	Control	Olfactory tract (both)	3	0
13	79	F	Control	Olfactory bulb + tract (right)	2	0
				Olfactory bulb + tract (left)	4	0
14	79	F	Control	Olfactory bulb	1	0
				Olfactory bulb + tract	2	0
15	80	M	Control	Olfactory bulb + tract	2	0
16	84	F	Control	Olfactory bulb + tract (both)	4	0
17	51	M	COVID19+	Olfactory bulb + tract (right)	1	0
				Olfactory bulb + tract (left)	2	0
18	52	M	COVID19+	Olfactory bulb	2	0
				Olfactory tract	2	0
19	56	M	COVID19+	Olfactory bulb + tract (right)	1	0
				Olfactory bulb + tract (left)	0	0
20	59	M	COVID19+	Olfactory bulb + tract (right)	2	0
				Olfactory bulb + tract (left)	6	0
21	61	F	COVID19+	Olfactory bulb + tract (right)	3	0
				Olfactory bulb + tract (left)	2	0
22	63	F	COVID19+	Olfactory bulb + tract (right)	4	0
				Olfactory tract (left)	4	0
23	73	F	COVID19+	Olfactory bulb + tract (right)	2	0
				Olfactory bulb + tract (left)	1	0
24	76	M	COVID19+	Olfactory bulb + tract (right)	5	0
				Olfactory bulb + tract (left)	5	0
25	76	F	COVID19+	Olfactory bulb + tract (right)	1	0
				Olfactory bulb + tract (left)	3	0

**Supplemental Table 7 cont'd**

Case ID	Age	Sex	Group	Tissue	# of AON	AON score
26	77	F	COVID19+	Olfactory tract (both)	4	0
27	78	M	COVID19+	Olfactory bulb + tract (both)	5	0
28	78	F	COVID19+	Olfactory bulb (right)	1	0
				Olfactory bulb (left)	1	0
29	79	M	COVID19+	Olfactory bulb + tract (right)	4	0
				Olfactory bulb + tract (left)	4	0
30	80	F	COVID19+	Olfactory bulb + tract (right)	1	0
				Olfactory bulb + tract (left)	1	0
31	83	F	COVID19+	Olfactory bulb + tract (right)	2	0
				Olfactory bulb + tract (left)	2	1
32	84	M	COVID19+	Olfactory bulb + tract (both)	3	1
33	84	M	COVID19+	Olfactory bulb + tract (right)	2	0
				Olfactory bulb + tract (left)	2	0
34	85	M	COVID19+	Olfactory bulb + tract (right)	2	0
				Olfactory bulb + tract (left)	3	2
35	86	M	COVID19+	Olfactory tract (right)	4	0
				Olfactory bulb + tract (left)	2	0
36	86	F	COVID19+	Olfactory bulb + tract (right)	2	0
				Olfactory bulb + tract (left)	2	0
37	89	M	COVID19+	Olfactory bulb + tract (right)	3	0
				Olfactory bulb + tract (left)	1	0
38	89	M	COVID19+	Olfactory bulb + tract (both)	3	0
39	72	M	LBD	Olfactory bulb + tract	5	5
40	73	M	LBD	Olfactory tract	0	0
				Olfactory bulb	3	4
41	77	M	LBD	Olfactory bulb	3	3
42	79	M	LBD	Olfactory bulb + tract	2	5
43	82	M	LBD	Olfactory bulb + tract	2	4
				Olfactory bulb	3	5
44	90	M	LBD	Olfactory tract	2	3
45	52	F	AD	Olfactory bulb + tract (both)	6	4
46	78	F	AD	Olfactory bulb + tract	2	0
				Olfactory bulb + tract	3	0
47	79	M	AD	Olfactory bulb + tract	2	0
				Olfactory bulb + tract	2	0
48	74	M	OND	Olfactory bulb + tract	4	0
49	79	M	OND	Olfactory tract (both)	2	0
50	81	M	OND	Olfactory tract	1	0

AON, anterior olfactory nucleus; LBD, Lewy Body disease; AD, Alzheimer's disease; OND, other neurodegenerative disease