

RESEARCH ARTICLE

Olfactory decline tracks central-to-peripheral spread of tau pathology in Alzheimer's disease

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Funding information

Fondation Louvain; StopAlzheimer
 Foundation; FRFS-Welbio; Télévie-FNRS;
 Fonds De La Recherche Scientifique - FNRS,
 Grant/Award Number: CCL40010417;
 UCLouvain

Abstract

INTRODUCTION: Olfactory decline often precedes cognitive symptoms in Alzheimer's disease (AD) and is linked to tau pathology. Yet, whether tau aggregates start in peripheral olfactory structures or spread from central regions remains debated.

METHODS: We analyzed 34 *post mortem* human olfactory bulb (OB) and 2 neuroepithelium (ONE) samples across Braak stages, combined with ex vivo nasal swabs and standardized olfactory testing in 88 clinically characterized participants.

RESULTS: Tau aggregates appeared in the anterior olfactory nucleus layer of the OB from Braak stage III, spreading to peripheral layers only in late stages. Olfactory identification declined in the preclinical phase and was linked to central tau pathology. Discrimination worsened during the prodromal stage, while threshold impairment appeared only in dementia, reflecting the anatomical progression of tau pathology.

DISCUSSION: This anatomical–functional link supports a central-to-peripheral spread of tau pathology in the olfactory system, with stage-specific deficits suggesting targeted smell tests as early AD biomarkers.

KEYWORDS

Alzheimer's disease, nasal brushing, olfaction, olfactory bulb, olfactory neuroepithelium, tau pathology

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Highlights

- Tau aggregates appear in the internal anterior olfactory nucleus layer of the human olfactory bulb (OB) at Braak stage III.
- The olfactory nerve layer of the OB and the olfactory neuroepithelium become involved only at advanced Braak stages.
- Olfactory subdomain impairments mirror the central-to-peripheral progression of tau pathology.
- Specific odor identification is impaired as early as the preclinical stage.

1 | BACKGROUND

Alzheimer's disease (AD) is a mixed proteinopathy characterized by extracellular amyloid plaques and intracellular neurofibrillary tangles (NFTs) composed of hyperphosphorylated tau.^{1,2} Both pathologies contribute to neurodegeneration and cognitive impairment,¹ but NFT burden shows a closer correlation with cognitive decline^{1,3,4} and more strongly predicts symptom onset.^{2,5-7} NFTs emerge through pathological tau transformation, in which excessive phosphorylation induces conformational changes that promote misfolding, oligomer formation, and subsequent aggregation.⁸ These oligomers progressively assemble into paired helical filaments,¹ which first appear as neuropil threads within axons and later evolve into pre-tangles in the perinuclear cell body.⁹ With disease progression, these structures mature into NFTs that disrupt neuronal integrity, impair synaptic communication, and compromise network function.³ Even after neuronal death, ghost tangles persist,⁹ highlighting their stability and underlining the role of tau aggregation as a progressive and self-perpetuating driver of AD pathology.

Tau pathology, widely regarded as the most reliable marker of AD progression, is believed to originate early in life in the locus coeruleus, later spreading to the transentorhinal cortex during Braak stage I.¹⁰ By Braak stage II, the olfactory bulb (OB), entorhinal cortex (EC), and hippocampus begin to exhibit pathological changes.¹⁰ Notably, during Braak stages I and II, cognitive functions remain intact. However, olfactory dysfunction often arises in AD before the onset of overt cognitive impairment and mostly impacting odor identification ability.¹¹⁻¹⁶ This olfactory decline has been linked to pathological tau aggregates in the EC at early stages, and in the OB, more specifically in the anterior olfactory nucleus (AON) layer, around Braak stages II to III.¹⁷⁻²⁰

Despite the established correlation between olfactory dysfunction and AD progression, it is still unclear whether the olfactory system plays a primary role in the development of the disease. The "olfactory vector hypothesis" proposes that the olfactory system may serve as an entry point for environmental or infectious agents that trigger cortical neurodegeneration.²¹⁻²³ Supporting this model, preventing the accumulation of pathological forms of tau in the olfactory neuroepithelium (ONE) through its recurrent stripping in the mouse model expressing the human P301S mutant form of tau, markedly reduces pre-tangle-like tau pathology in the piriform cortex.²⁴

In humans, evidence for early tau pathology in the ONE is limited and inconsistent. A few studies have reported tangle-like profiles in the ONE tissue of AD cases,²⁵⁻²⁷ yet these observations were made predominantly in patients at advanced stages of the disease. Conversely, some studies reported little to no tau labeling in pathological conditions, with occasional staining also observed in non-AD or control individuals.²⁸⁻³¹ Contrasting conclusions drawn by these studies contribute to the uncertainty surrounding whether the human ONE exhibits tau pathology, particularly at the earliest stages of AD. Moreover, a recent study convincingly demonstrated tau propagation from medial temporal lobe structures toward the peripheral olfactory system,³² challenging the theory that tau pathology occurs in the ONE due to infectious or environmental agents and eventually spreads to connected region. These opposing hypotheses nevertheless underscore the complexity and potential bidirectionality of tau spreading within the olfactory pathway, as well as possible differences between humans and mice.

Determining whether tau pathology originates in the olfactory cortex or peripheral olfactory structures is crucial for developing early interventions for AD. The primary objective of this study was to test the directionality of tau pathology propagation within the human olfactory system. To address this objective, we combined histopathological analyses of *post mortem* olfactory tissues with *ex vivo* nasal epithelial samples from individuals spanning the AD clinical continuum, together with olfactory assessments. This integrated approach enabled us to map the anatomical progression of tau pathology, supporting a trajectory from the central nervous system to peripheral olfactory structures. It also underscored the value of combining biological and functional measures to capture early disease dynamics.

2 | METHODS

2.1 | *Post mortem* human samples

Human *post mortem* OB tissues were obtained from the Netherlands Brain Bank (NBB), with anonymized clinical summaries of the donor's medical record and histopathological brain analysis (Table S1 in supporting information). Two *post mortem* human OB tissues from body donors at Braak stage II were obtained from UCLouvain body dona-

tion program (ethical approval: UCL-2020-355; Table S1). In total, OB samples from 34 patients were used ($F = 20$, $M = 14$; 81.0 ± 15.1 years). All presented tauopathy ranging from Braak stage I to Braak stage VI (I: $n = 3$, II: $n = 5$, III: $n = 5$, IV: $n = 5$, V: $n = 4$, VI: $n = 12$). Braak staging was determined by the brain banks according to a widely accepted neuropathological standard³³ based on phosphorylated tau (p-tau) immunohistochemistry (IHC) using the AT8 antibody (P-tau Ser202/Thr205). Assessments were performed by a single rater on sections from the hippocampus and EC, temporal associative cortex, and occipital cortex. Braak stages were interpreted as a continuum reflecting the progressive spread of NFTs from transentorhinal regions to the occipital cortex, with hippocampal involvement generally observed from stage II onward. For 15 patients, both frozen and fixed tissues were available; for 17 patients, only frozen tissues were available; and for 2 patients, only fixed tissues were obtained (Table S1). Frozen bulb samples were mechanically homogenized and split into two fractions for different analyses. Two *post mortem* human ONE from body donors at Braak stage II were also obtained from UCLouvain body donation program ($F = 1$, $M = 1$; 92.5 ± 2.5 years, ethical approval: UCL-2020-355).

2.2 | Participants recruitment for ex vivo study

Participants were recruited through clinical neurology consultations and from clinical studies led by Prof. Bernard Hanseeuw and Prof. Caroline Huart at Cliniques Universitaires Saint-Luc, the academic hospital of UCLouvain. This study was performed according to the Declaration of Helsinki and approved by the medical ethics committee of the academic hospital of UCLouvain (ethical approvals: UCL-2016-121 and UCL-2010-148). The 88 participants ($F = 45$, $M = 43$) provided written informed consent (Table S2 in supporting information). An oral questionnaire was run to exclude individuals meeting predefined exclusion criteria: known cause of olfactory disorder, acute or chronic rhinosinusitis, history of head trauma, participation in a clinical trial involving an AD treatment, or a medical history of major psychiatric conditions or other neurological disorders. The participants' diagnoses described below relied on an examination of amyloid and tau status with either tau positron emission tomography (PET) imaging with [¹⁸F]-MK6240 plus 3D T1 magnetic resonance imaging (MRI; $n = 37$),³⁴ amyloid PET ($n = 57$), cerebrospinal fluid (CSF) analyses ($n = 19$), or blood plasma analyses ($n = 9$). Clinical diagnosis were based on the Mini-Mental State Examination (MMSE; $n = 88$) and a neuropsychological examination ($n = 57$), which included testing of four cognitive domains (verbal episodic memory, executive function, language, and visuospatial function), described in the supplementary Methods and Table S3 in supporting information.

2.3 | Evaluation of amyloid and tau status

Amyloid positivity was determined by either CSF analysis, blood analysis, or by amyloid PET with [¹⁸F] flutemetamol or [¹¹C] Pittsburgh

RESEARCH IN CONTEXT

- Systematic review:** The prevailing model of tau pathology propagation in Alzheimer's disease (AD), based on Braak staging, posits a "prion-like" mechanism characterized by progressive transsynaptic spread from central brain regions to more peripheral cortical areas. Because olfactory deficits frequently precede cognitive symptoms, some have proposed that tau pathology may originate in peripheral regions, such as the olfactory neuroepithelium, and spread centrally toward the olfactory bulb and cortex. This theory is supported by the fact that the olfactory neuroepithelium is directly exposed to environmental stressors, including toxins and pathogens. However, this view has been challenged by studies demonstrating a trajectory consistent with central-to-peripheral spread.
- Interpretation:** In this study, we combined cellular, molecular, and functional approaches to determine whether the olfactory system is a primary target or a secondary site of involvement in the progression of AD. Our analysis encompassed *post mortem* human olfactory tissues and ex vivo nasal epithelial cells from individuals across different clinical stages, together with corresponding assessments of olfactory function. Our results support a retrograde model of tau pathology propagation, from the olfactory cortex to the olfactory neuroepithelium via the olfactory bulb. These results challenge the notion that the human olfactory epithelium serves as the initial site of tau pathology onset.
- Future directions:** This work supports the considerable potential of olfactory testing in AD, both for improving diagnosis and for refining disease staging and monitoring.

compound B as described previously.^{35,36} PET values were expressed on the Centiloid scale; blood plasma amyloid beta ($A\beta$)42/ $A\beta$ 40 and CSF $A\beta$ 42 levels were measured with automated assays.³⁵⁻³⁷ Participants were classified as amyloid positive (A+) if Centiloid > 26, plasma soluble $A\beta$ 42/ $A\beta$ 40 ratio ≤ 0.0465 , or CSF $A\beta$ 42 < 437 pg/ml.^{36,38-40} Tau pathology was assessed with [¹⁸F]MK-6240 PET (Lantheus Inc.), a second-generation tracer for tau tangles as described previously.⁴¹ Radiosynthesis was performed at KULeuven and doses of 185 ± 5 MBq were administered intravenously. Ninety minutes post-injection, a 30-minute dynamic acquisition was performed on a Philips Vereos digital PET CT. Images were reconstructed with the manufacturer's algorithm (attenuation, scatter, decay correction, time of flight, point spread function modeling, and 1 mm reslicing). Tau PET scans were co-registered with a 3D T1 MRI for visual analysis and the Braak-like stage was reported for each participant. Participants were classified as tau positive (T+) if the visual Braak-like stage was > 0.

TABLE 1 Individuals enrolled in the study for nasal brushings and olfactory tests.

Category	A– CN	A+ CN	Prodromal AD	AD dementia
Number of participants	29	15	28	13
Mean age (years)	70.4	73.6	70.8	75.2
Sex (F:M)	(15:14)	(8:7)	(13:15)	(8:5)
Amyloid testing (A)	A–	A+	A+	A+
Tau testing (T)	T–	T– or T+	T+	T+
Global cognitive Z score (SD)	≥ –1.5	≥ –1.5	< –1.5	< –1.5
MMSE (/30)	> 24	> 24	> 24	≤ 24

Notes: Summary of demographic and diagnostic data for individuals enrolled in the study, including clinically normal participants and patients at preclinical, prodromal, or advanced stages of dementia. Diagnoses were based on comprehensive clinical evaluation, neuropsychological testing, brain imaging (positron emission tomography), blood samples and cerebrospinal fluid analysis when available.

Abbreviations: AD, Alzheimer's disease; CN, cognitively normal; MMSE, Mini-Mental State Examination; SD, standard deviation.

2.4 | Clinical classification of participants

Participants were classified as either cognitively normal (CN; $n = 47$, all cognitive domain z scores ≥ -1.5 and MMSE > 24) or cognitively impaired (CI; $n = 41$, at least one cognitive domain z score < -1.5). Among CN participants, 44 had available amyloid testing and were further subdivided into two subgroups with either normal ($n = 29$, A–) or abnormal ($n = 15$ A+) amyloid status, while 3 CN participants had no available amyloid or tau biomarker data. The A+ CN group comprises preclinical AD patients with either normal ($n = 8$ T–) or abnormal ($n = 7$ T+) tau PET. To focus specifically on AD, only A+ CI individuals were included. A+ CI participants were further subdivided in two subgroups based on disease severity with either prodromal AD (PR AD; $n = 28$) or AD dementia ($n = 13$; Table 1 and S2).

2.5 | Olfactory tests

Olfactory tests were performed using the Sniffin' Sticks test (Burghart) validated by Hummel et al.⁴² This test includes three subtests yielding three scores: threshold (T), discrimination (D), and identification (I). Odors were delivered using pen-like devices with tips infused with an odorant solution. The tip was held 2 cm below the nose and moved slowly from the left to right nostril for > 3 to 4 seconds. The threshold test consisted of randomized triplets of pens numbered 1 to 16. In each triplet, only one pen contained the odorant (phenylethanol), with pen no. 1 containing the most concentrated solution. The odorant was serially diluted (1:2) down to pen no. 16. For each triplet, the subject was asked to identify the odor-containing pen in a three-alternative forced-choice (3-AFC) paradigm with eyes closed. The test starts with the lowest concentration, and a staircase paradigm is used, by which two consecutive correct answers led to a lower concentration in the next triplet and one incorrect answer led to a higher concentration in the next triplet. This procedure is repeated until seven turning points are identified. The T score was the mean of the last four turning points in the staircase, ranging from 1 to 16. The discrimination test presented 16 triplets in random order: two pens contained the same odor, while

the third contained a different odor. Subjects identified the different odor in a 3-AFC paradigm, eyes closed. The D score was the number of correct responses (0–16). Sixteen pens were presented individually; for each, subjects were asked to identify the odor based on a four-alternative forced choice procedure from four illustrated options read aloud by the examiner. The I score was the number of correct identifications (0–16). The final TDI score was the sum of the three individual scores (range 1–48).

2.6 | Nasal brushing and cell isolation

Nasal brushing was performed by a trained ear, nose, and throat specialist using two flocked swabs (Copan, FLOQSwabs 503CS01) introduced into both olfactory clefts at the level of the middle turbinate under endoscopic guidance. The ONE was exfoliated from both nostrils by rotation. One swab was placed in 1 mL of 4% paraformaldehyde; the other in 1 mL chilled Dulbecco's Modified Eagle Medium/F12 (Thermo Fisher Scientific, Gibco 11039021) with 10% fetal bovine serum (Cytiva, SV30160.03), 1% penicillin/streptomycin solution (Capricorn Scientific, PS-B), protease inhibitors (1:25; Roche, 11836170001), and phosphatase inhibitors (1:20; Roche, 4906837001). After vortexing for 15 seconds, the swab was removed from the fixative. The cell-containing solution was centrifuged 15 minutes at $300 \times g$, fixative discarded, pellet resuspended in phosphate-buffered saline (PBS), and cytocentrifuged (Cytospin 4, Thermo Fisher Scientific) for 10 minutes at $200 \times g$ onto slides (Immuno-Cell, 61.100.59). The swab in culture medium was rubbed against another swab to detach cells. Samples were then divided into two aliquots before centrifugation at 4°C for 45 seconds at $20,630 \times g$. Supernatants were removed and cell pellets were frozen at -80°C .

2.7 | IHC

Ten-micrometer sections of paraffin-embedded *post mortem* OB tissues were prepared using a microtome (Microm Microtech, HM 340E).

The sections were deparaffinized by incubating them in two successive xylene baths (5 minutes each), followed by two 2-minute immersions in 100% isopropanol. Endogenous peroxidases were inactivated in 0.3% H₂O₂/methanol for 30 minutes. After rinsing with distilled water and delineation of tissue areas using a DakoPen (Sigma-Aldrich, Z377821), sections were washed three times for 10 minutes in Tris-buffered saline (TBS), then blocked for 1 hour at room temperature (RT) in 10% normal goat serum (NGS; Sigma-Aldrich, S26-M) and incubated overnight with primary antibodies (Table S4 in supporting information). Secondary antibody incubation was carried out for 30 minutes at RT. Negative controls omitted primary antibodies. Sections were washed three times for 10 minutes in TBS, incubated with streptavidin-horseradish peroxidase (Vector Laboratories, SK-4103) for 30 minutes, rinsed three times 10 minutes, and developed with 3,3'-diaminobenzidine (Vector Laboratories, PK-7100): 2 minutes for PHF1/AT8, 1 minute 30 seconds for AT270/olfactory marker protein (OMP). Reactions were stopped in distilled water. Sections were dehydrated through graded isopropanol baths (70%–100%, 4 × 2 minutes) and finally mounted with distyrene, plasticizer, and xylene (Sigma-Aldrich, 1.00579.0500). Visualization was conducted using a ZEISS Axioskop 40 microscope; images were captured with ZEISS ZEN Blue 2.5 and analyzed in ImageJ2.⁴³

2.8 | Immunofluorescence

For IF experiments, *post mortem* OB paraffin blocks were sectioned at 5 µm. Sections were deparaffinized and rehydrated through a series of xylene, isopropanol, ethanol, and distilled water washes, then rinsed in 1 × PBS. Antigen retrieval was performed by heating in boiling citrate buffer (10 mM, 0.1% Tween, pH 6) for two 5-minute cycles, followed by a 20-minute cooldown at RT. Permeabilization was performed in Tris-HCl buffer with 0.5% Triton X-100 for 1 hour at 37°C. After 1 × PBS rinses, non-specific binding was blocked for 45 minutes at RT using 1 × PBS supplemented with 0.1% Triton X-100, 0.21% bovine serum albumin (BSA) and 2.1% NGS. For *ex vivo* nasal brushing cells, antigen retrieval was performed with two successive incubations in boiling citrate buffer (1 minute followed by 3 minutes), followed by 30 minutes of permeabilization at RT. Blocking was then carried out for 1 hour at RT in 1 × PBS containing 0.1% Triton X-100, 0.21% BSA, and 2.1% normal horse serum (NHS). Samples were incubated overnight at 4°C with primary antibodies (Table S4). Negative controls omitted primary antibodies. The next day, samples were washed and incubated for 40 minutes at RT with secondary antibodies (Table S4). After final washes with 1 × PBS, slides were mounted in fluorescence mounting medium (Agilent, S3023) containing 0.1 µg/mL 4',6-diamidino-2-phenylindole (Sigma-Aldrich, D9542). Visualization was done using a ZEISS Axio Observer Z1 confocal microscope; images were acquired with ZEISS ZEN Blue 2.6 and analyzed in ImageJ2.⁴³ Low-magnification (2X) images were obtained with a Panoramic Confocal scanner (3DHISTECH).

2.9 | Enzyme-linked immunosorbent assay

Post mortem OB and *ex vivo* smear samples were lysed in a buffer containing 150 mM NaCl, 20 mM Tris, 1% NP-40 (Sigma, 74385-1L), and 10% glycerol, supplemented with protease inhibitors (1:25, Roche, 11836170001) and phosphatase inhibitors (1:20, Roche, 4906837001). Mechanical lysis was performed using a syringe (Terumo Agani, 15438652; BD Plastipak, 303172). After centrifugation for 10 minutes at 20,000 × g at 4°C, the supernatant was collected and protein concentration was determined using the Bradford assay. P-tau at Thr181 (p-tau181) and total tau were quantified using commercial sandwich enzyme-linked immunosorbent assay (ELISA) kits (Thermo Fisher Scientific, KHO0631; KHB0041) per manufacturer's instructions. All assays were run in duplicate. Protein input was maximized, averaging 20 µg/well. Detection limits were 15.6 pg/mL for p-tau181 and 31 pg/mL for total tau. P-tau181 levels were normalized to total tau for each sample.

2.10 | Statistical analyses

Figures and statistical analyses were performed using GraphPad Prism 10. Normality was evaluated using the Shapiro–Wilk test ($\alpha = 0.05$). For two-group analyses, a Student *t* test was used when normality was met; otherwise, the Wilcoxon test was applied. For multiple group comparisons, parametric one-way analysis of variance was used for normally distributed data, otherwise the Kruskal–Wallis test was applied.

3 | RESULTS

3.1 | NFT-like aggregates accumulate in the *post mortem* OB from Braak stage III onward

In the OB and AON, a relay structure that connects the bulb to the olfactory cortex, NFTs and neuropil threads have been previously reported to emerge from Braak stage II to III onward.^{17,18} Accordingly, using IHC, we observed small amounts of NFT-like aggregates in all 13 *post mortem* OB samples from donors with Braak stage III or higher, with their abundance progressively increasing at more advanced stages (Figure 1A and Figure S1 in supporting information). The flame-shaped aggregates characteristic of NFTs were, however, not observed in OB samples from patients at Braak stage I or II (Figure 1A and Figure S1). Similar observations were made with the PHF1 antibody (P-tau Ser396/Ser404), a later-stage marker more specific to the mature forms of pathological aggregates and specifically highlighting flame-shaped NFT-like aggregates (Figure 1A).

Consistent with previous studies,^{17,44–48} we observed that, from Braak stage III onward, NFTs accumulate in the AON layer of the OB, which we identified using an anti-calbindin antibody, a marker of interneurons primarily located in the AON layer (Figure 1B). In contrast, the outer layer of the OB, known as the olfactory nerve layer

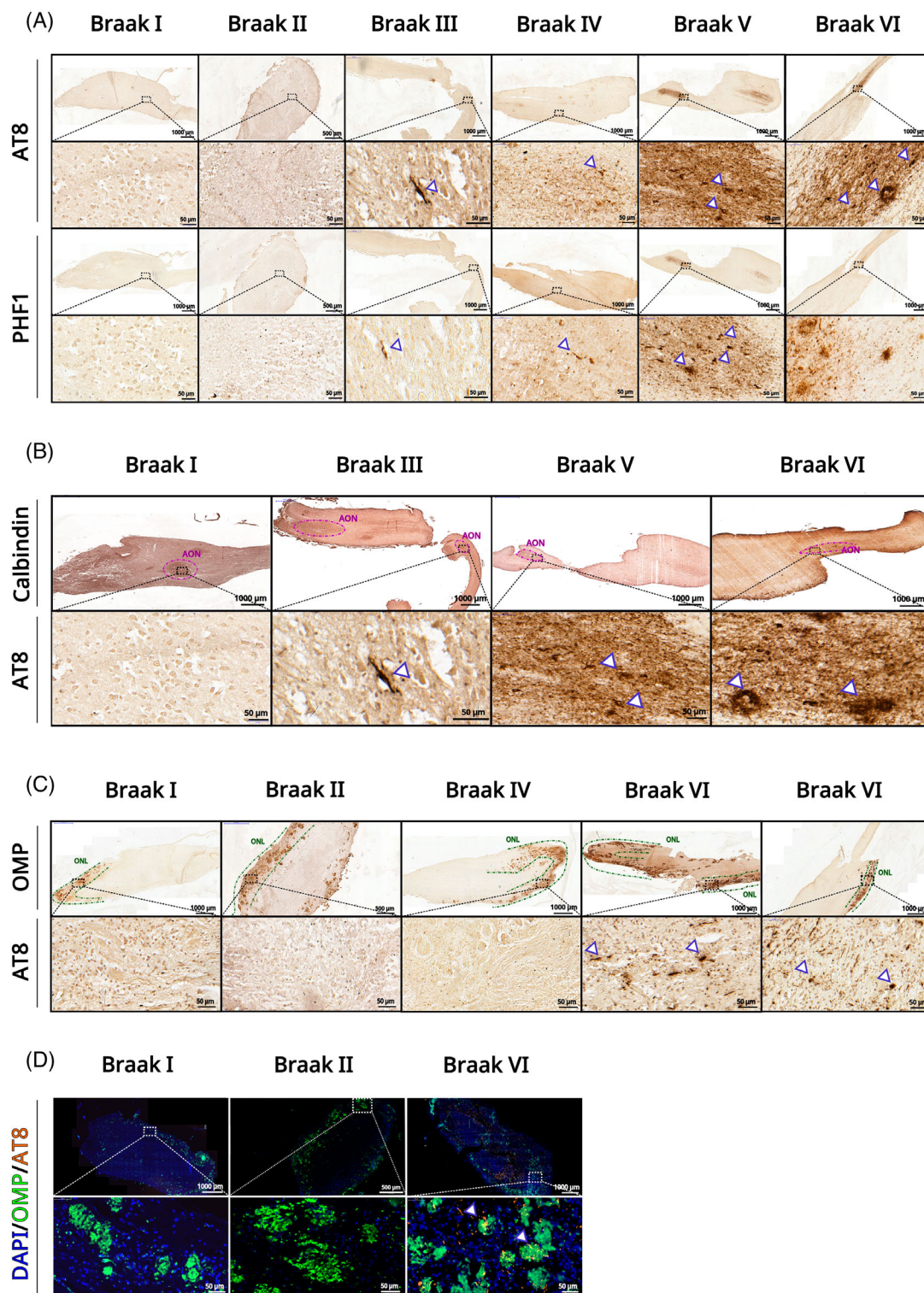


FIGURE 1 Distribution of NFT-like aggregates in human olfactory bulbs across Braak stages. A, Representative pictures of paraffin sections immunostained for AT8 (Ser202/Thr205) or PHF1 (Ser396/Ser404) in OB samples from patients at the indicated Braak stages (n = 6). Above: Low magnification view, scale bar: 500 or 1000 μm, as indicated. Below: Higher magnification of the highlighted region, scale bar: 50 μm. Flame-shaped NFTs (arrowheads) were not observed at Braak stages I or II. B, Above: The AON region in the OB samples (n = 4) was identified using calbindin immunolabeling (purple dotted lines), scale bar: 1000 μm. Below: Higher magnification of the highlighted region, scale bar: 50 μm. Arrowheads indicate NFT-like aggregates. Co-detection of the ONL and NFTs in OB samples (n = 5) from donors at indicated Braak stage using OMP and AT8 antibodies by IHC (C) or IF (D) (n = 3; lower panel with OMP in green, AT8 in red, and DNA in blue). Above: Low magnification view, scale bar: 500 or 1000 μm, as indicated. Below: Higher magnification of the highlighted region, scale bar: 50 μm. Co-localization events are indicated by arrowheads. AON, anterior olfactory nucleus; IF, immunofluorescence; IHC, immunohistochemistry; NFT, neurofibrillary tangle; OB, olfactory bulb; OMP, olfactory marker protein; ONL, olfactory nerve layer.

(ONL), identified using an antibody against the OMP, remained unaffected at Braak stage IV (Figure 1C). However, AT8-positive NFT-like aggregates appeared by stage VI, indicating a delayed onset of NFTs in the ONL compared to the AON layer in AD patients (Figure 1D).

Together, our findings support the idea that although NFTs emerge early in the AON layer, their accumulation in the ONL happens during the advanced stages of the disease.

3.2 | P-tau181-positive deposits accumulate in the AON from Braak stage I onward

Having found that NFTs appear in the OB of AD patients from Braak stage III, we next assessed the spatiotemporal progression of pathological tau filaments known as neurofibrillary threads, specifically labeled by the AT270 antibody targeting p-tau181, a modification widely used as a quantifiable biomarker in human CSF and known to appear earlier in the progression of tau pathology than p-tau205.⁴⁹ We detected tau deposits possibly corresponding to neurofibrillary threads in the AON layer as early as Braak stage I, with a progressive increase in their accumulation at advanced stages (Figure 2A). Notably, the early distribution of these aggregates appeared to be layer specific. Specifically, in Braak I samples in which the earliest neurofibrillary threads were observed in the inner layers of the olfactory bulb, we also noted the absence of these aggregates in the OMP-positive layers corresponding to the ONL (Figure 2B). These findings support the notion that tau aggregation, from the formation of neurofibrillary threads to the emergence of the first NFTs, initially develops in inner layers such as the AON before extending to more peripheral layers such as the ONL. Consistent with the AT8 and PHF1 stainings however, NFT-like aggregates only became detectable with this antibody at Braak stage V and were found clustering in the AON layer (Figure 2A). The results obtained by immunostaining were confirmed by ELISA as we found a significant increase in the p-tau181/total tau ratios at advanced Braak stages (V/VI) compared to early stages (I/II; Figure 2C).

Together, this spatiotemporal progression of tau immunoreactivity across Braak stages supports a model in which the pathology progresses from the AON layer to the rest of the OB.

3.3 | NFT-like lesions are not detected in exfoliated cells from the ONE in living humans

To further investigate the progression of tau pathology within the olfactory system, we next examined the presence of NFTs in the ONE. Previous studies on AD pathology in the ONE have primarily examined *post mortem* tissues from individuals with late-stage dementia, limiting the ability to assess the presence of tau pathology in the ONE at earlier Braak stages.^{27,28} Due to the limited availability of *post mortem* ONE tissues, we adopted an *ex vivo* approach, analyzing exfoliated cells collected from nasal brushing of living individuals. These samples were collected from a cohort representing the full clinical spectrum of AD, including amyloid-negative CN individuals (A– CN, $n = 29$), as well as patients at preclinical stage, CN, but with amyloid pathology (A+ CN,

$n = 15$). This cohort also included CI patients at the prodromal (PR AD, $n = 28$), or dementia (AD dementia, $n = 13$) stages of AD (Table 1 and S2).

First, to determine whether nasal smears contain mature olfactory sensory neurons, we performed immunofluorescence against OMP, using *post mortem* ONE tissue as a positive control for the staining (Figure 3A). Next, we tested a total of 26 nasal smear samples ($n = 9$ CN, $n = 9$ PR AD, and $n = 8$ AD dementia) and observed OMP staining in $\approx 11\%$ of the collected cells (Figure 3A), with a pattern closely resembling previously reported observations.⁵⁰ After validating the nasal brushing approach for studying the ONE, we proceeded to assess tau pathology. First, we confirmed the presence of total tau in the dendritic cilia of nasal smear cells, mirroring the distribution observed in the ONE tissue (Figure 3A). However, AT8 immunoreactivity was absent in OMP-positive neurons collected via nasal smear, even in individuals with dementia, suggesting that NFTs do not form in these cells (Figure 3B).

These findings indicate that NFT-like lesions do not form in olfactory sensory neurons, supporting the hypothesis that ONE is unlikely to serve as the entry point for tau pathology in humans.

3.4 | Increased levels of p-tau181 in nasal smears from patients with AD dementia

Given the apparent lack of NFT-like lesions in nasal brushing, even in dementia patients, we next examined the samples for potential differential enrichment of p-tau181. We first attempted to measure p-tau181 levels in nasal smear extracts using ELISA. While total tau was readily detectable, p-tau181 levels fell below the assay's detection threshold (Figure S2A–B in supporting information). We therefore turned to immunostaining, using *post mortem* ONE tissue from a Braak stage II case as a positive control (Figure 4A). To assess p-tau181 levels specifically in OMP⁺ cells within the nasal smears, we performed co-staining with AT270 (p-tau181) and OMP antibodies (Figure 4A). For each sample, the ratio of OMP⁺/p-tau181⁺ cells was compared to the ratio of OMP⁺/total tau⁺ cells (Figure 3A and 4A). Analysis of samples from CN ($n = 9$), PR AD ($n = 9$), and AD dementia patients ($n = 8$) revealed a pattern consistent with that observed in the OB: a significant increase in tau phosphorylation in AD dementia cases ($p = 0.037$), and a trend toward increased p-tau181 in the prodromal group ($p = 0.23$; Figure 4B).

Together, these data support the results obtained with the OB samples and the hypothesis that human tau pathology does not start in the ONE.

3.5 | Olfactory identification declines in the preclinical stage, while detection threshold drops only in AD dementia

Early impairment in odor identification has been previously linked to tau aggregates in primary olfactory regions such as the piriform cortex, AON, amygdala, and EC.³² It remains unclear whether threshold

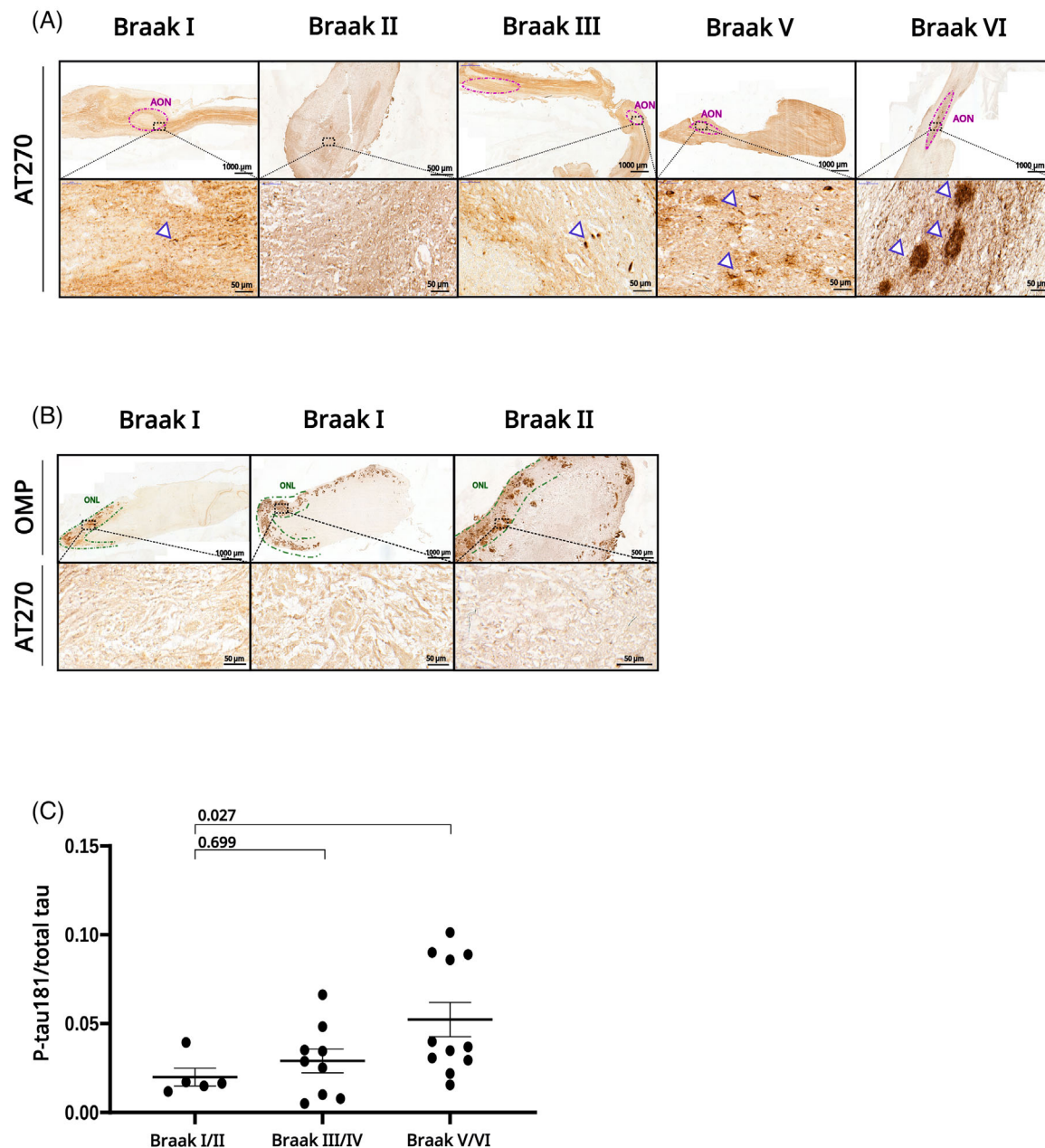


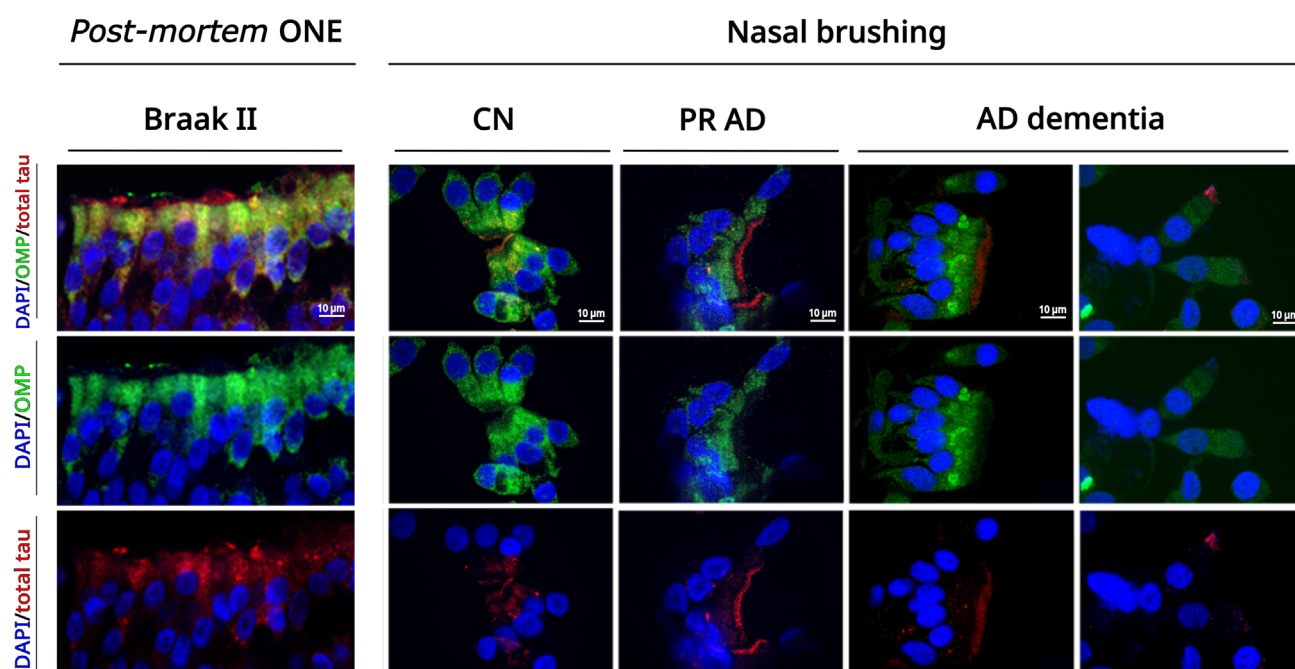
FIGURE 2 Detection of p-tau181 in human olfactory bulbs across Braak stages. A, Representative pictures of paraffin sections immunostained with the AT270 antibody (p-tau181). Above: Low magnification view, scale bar: 500 or 1000 μm , as indicated. The position of the AON is indicated with purple dotted lines. Below: Higher magnification of the highlighted region, scale bar: 50 μm . Tau deposits appear as early as Braak stage I while NFT-like aggregates become detectable only from Braak stage V onward (arrowheads) in the OB samples ($n = 5$). B, Co-detection of the ONL and neuropil threads aggregates in OB samples ($n = 3$) from donors at indicated Braak stage using OMP and AT270 antibodies by IHC. Above: Low magnification view, scale bar: 500 or 1000 μm , as indicated. Below: Higher magnification of the highlighted region, scale bar: 50 μm (C) Quantification of p-tau181 and total tau levels by enzyme-linked immunosorbent assay in OB tissue extracts from patients at the indicated Braak stages ($n = 25$). Mean $\pm$ standard error of the mean, Mann-Whitney statistical test. AON, anterior olfactory nucleus; IHC, immunohistochemistry; NFT, neurofibrillary tangle; OB, olfactory bulb; OMP, olfactory marker protein; ONL, olfactory nerve layer; p-tau, phosphorylated tau.

performance—generally considered an indicator of peripheral olfactory function—is impacted in the early stages of AD pathology.⁵¹ To investigate this further and to gain additional insight into the direction of tau pathology spread, we evaluated olfactory function using the standardized Sniffin' Sticks test in an age-matched cohort encompassing various clinical stages of AD (Table 1 and S2). This test evaluates

three distinct components of olfactory ability: identification (I), but also threshold (T) and discrimination (D), each with a maximum score of 16.

We found that the overall TDI score declined significantly at the prodromal ($p = 0.041$) and dementia ($p < 0.0001$) stage of AD compared to A–CN volunteers (Figure 5A). When analyzing the three subcomponents individually, the T score showed a significant decline

(A)



(B)

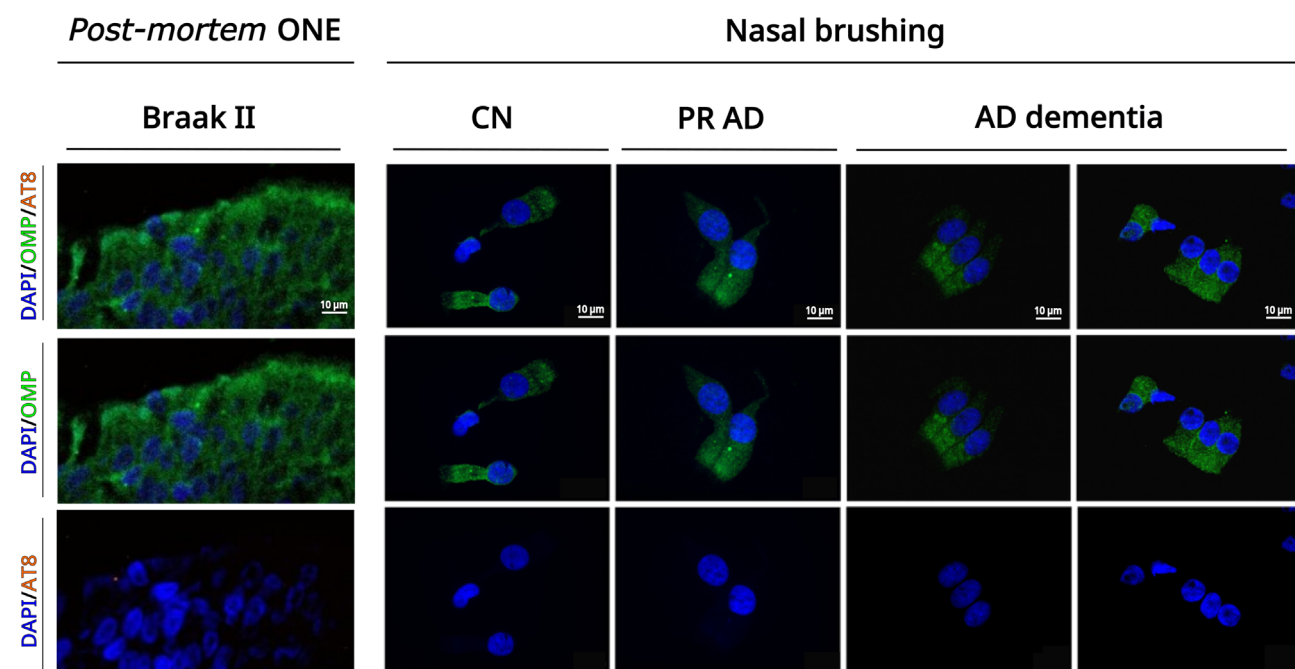


FIGURE 3 Detection of NFT-like aggregates in human olfactory neurons from olfactory neuroepithelium and ex vivo nasal smears. A, Representative pictures of immunofluorescence staining using OMP (green) and tau (red) antibodies. DNA, stained with DAPI (blue), marks nuclei. Left: *post mortem* ONE sample from a patient at Braak stage II ($n = 1$); right: representative pictures of nasal smear cells ($n = 4$) from CN donors or AD patients at the prodromal (PR AD) or the dementia stage of AD (AD dementia). Scale bar: 10 μ m. B, Same as (A) using OMP (green) and AT8 (red) antibodies. Scale bar: 10 μ m. AD, Alzheimer's disease; CN, cognitively normal; DAPI, 4',6-diamidino-2-phenylindole; OMP, olfactory marker protein; ONE, olfactory neuroepithelium.

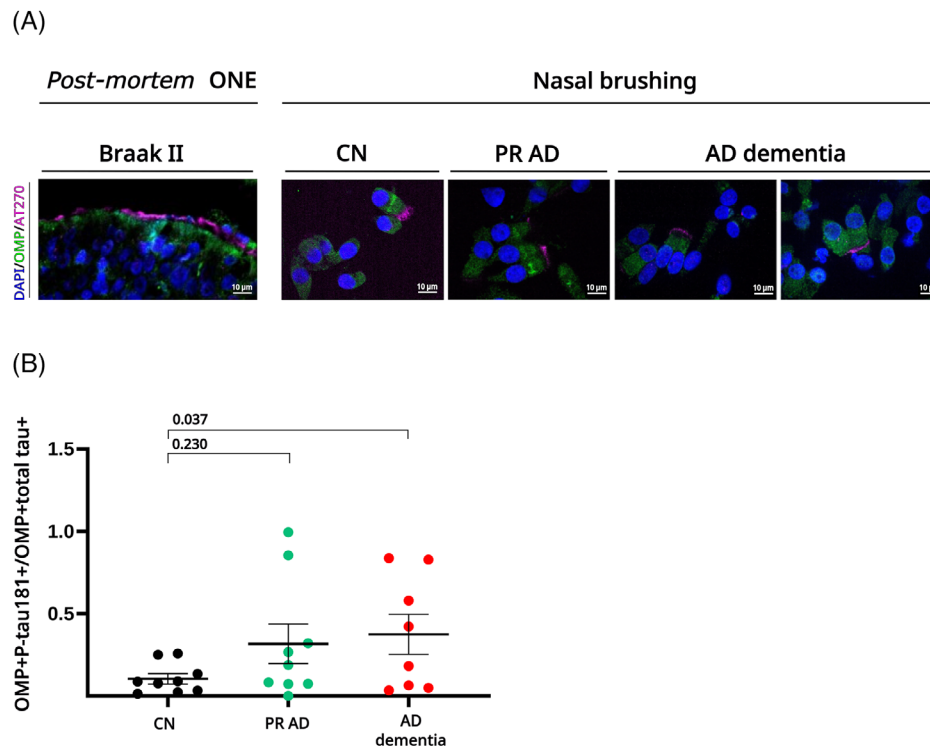


FIGURE 4 Detection of p-tau181 in human olfactory neurons from olfactory neuroepithelium and ex vivo nasal smears. A, Representative pictures of immunofluorescence staining using OMP (green) and AT270 (P-tau181) (red) antibodies. DNA, stained with DAPI (blue), marks nuclei. Left: *post mortem* ONE sample from a patient at Braak stage II ($n = 1$); right: representative pictures of nasal brushing cells ($n = 4$) from CN donors or AD patients at the prodromal (PR AD) or the dementia stage of AD (AD dementia). Scale bar: 10 μm . B, Quantification of the ratio of OMP⁺/p-tau181⁺ to OMP⁺/tau⁺ cells across CN participants ($n = 9$), prodromal AD patients ($n = 9$), and AD dementia patients ($n = 8$). Mean $\pm$ standard error of the mean, unpaired t , and Mann-Whitney statistical tests. AD, Alzheimer's disease; CN, cognitively normal; DAPI, 4',6-diamidino-2-phenylindole; OMP, olfactory marker protein; ONE, olfactory neuroepithelium; p-tau, phosphorylated tau.

only at the dementia stage ($p < 0.0001$; Figure 5B). In contrast, D ability was already significantly impaired at the prodromal stage ($p = 0.0125$; Figure 5C). Consistent with previous findings,^{15,16,52,53} the I score followed a different trajectory, with a significant reduction evident as early as the preclinical stage in A+ CN individuals ($p = 0.004$), and continued to decline through the prodromal and dementia stages (Figure 5D). A+ CN patients can be either negative or positive for tau pathology (Table 1). To further explore whether the early deficit in identification performance may be linked to tau pathology, we stratified A+ CN patients into two subgroups: those with a positive amyloid PET, but negative tau PET (A+T-; $n = 8$), and those with both amyloid and tau PET positivity (A+T+; $n = 7$). The results revealed a significant decline in identification scores exclusively in A+T+ CN patients compared to A- CN participants ($p < 0.0001$), supporting an early association between olfactory dysfunction and tau accumulation in primary olfactory regions (Figure 5E).

Consistent with the p-tau181 staining results, the decline in threshold performances was only seen at the dementia stage of AD, with no reduction at earlier stages (Figure 5B). This prompted us to test whether the late decline in threshold performances may be related to the increased OMP⁺/p-tau181⁺ to OMP⁺/tau⁺ ratio that we observed in nasal smear cells in late disease stages (Figure 4A–B). Consistently, we observed a trend toward an inverse correlation ($R^2 = 0.39$;

$p = 0.003$) between threshold scores and the ratio of OMP⁺/p-tau181⁺ to OMP⁺/tau⁺ cells in nasal smears (Figure 5F), whereas the correlation with discrimination scores was notably weaker ($R^2 = 0.15$; $p = 0.087$; Figure S3 in supporting information).

Together, these data support the hypothesis that early olfactory impairment in AD, marked by defective identification ability, stems from tau pathology in the central nervous system, which later spreads to the olfactory nasal epithelium and impacts detection threshold.

3.6 | Identification of orange odor is significantly impaired from the preclinical stage

Earlier work using the UPSIT (University of Pennsylvania Smell Identification Test) assay of odor identification has associated pathological tau accumulation in the brain with impaired identification of certain specific odors.³² Notably, the scent of orange was strongly associated with early tau deposition in olfactory regions during Braak stages II and III, while the scent of leather was linked to tau accumulation at Braak stages III to IV.³²

This prompted us to analyze participants' responses to each of the 16 odors in the Sniffin' Sticks identification test (Figure 6A). To focus

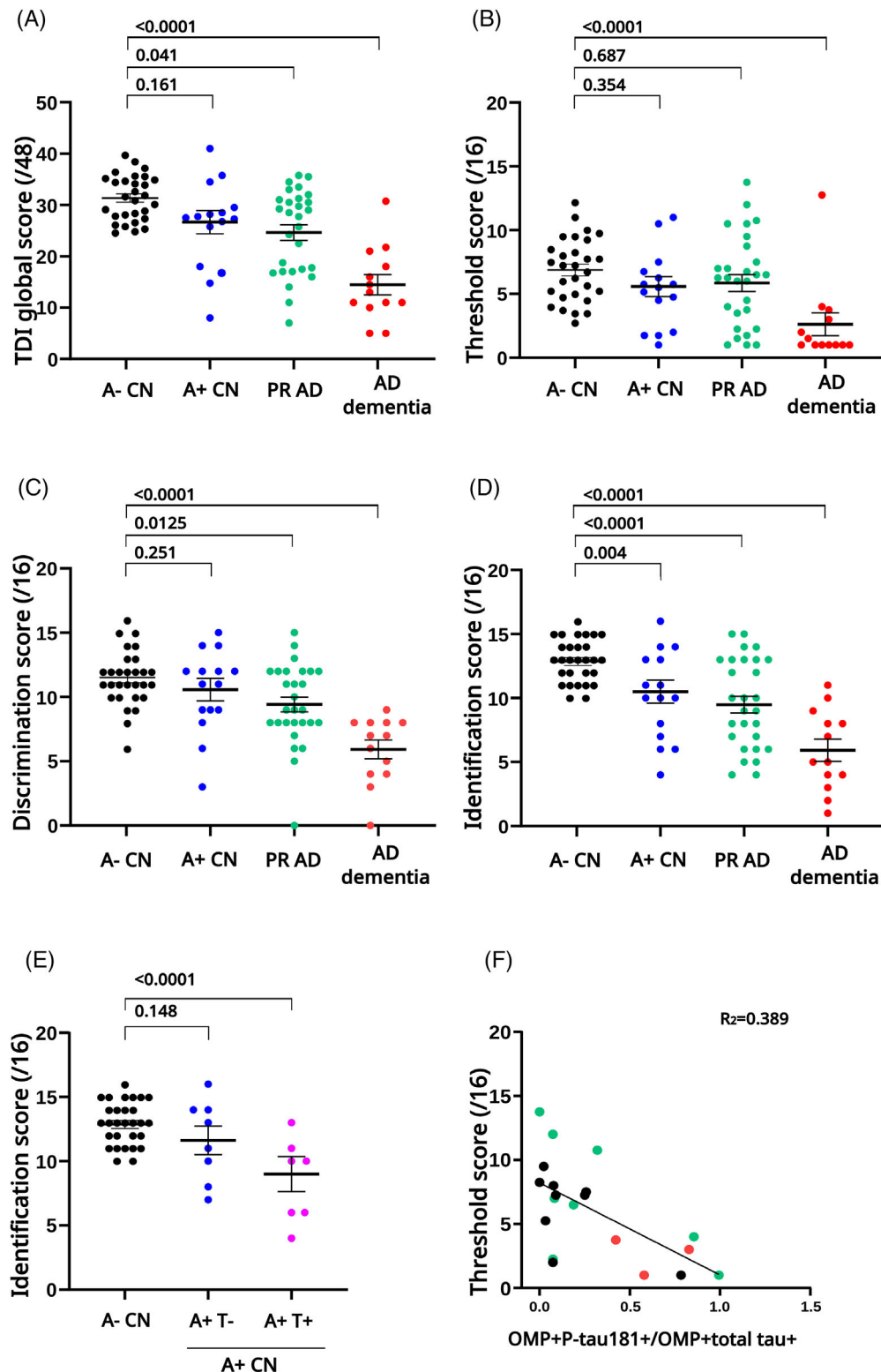


FIGURE 5 Olfactory dysfunction correlates with tau pathology across AD stages. A, The composite TDI score was determined using the standardized Sniffin' Sticks test in $n = 29$ A- CN ($F = 15$, $M = 14$; mean age = 70.4 years) and $n = 15$ A+ CN ($F = 8$, $M = 7$; mean age = 73.6 years) participants, $n = 28$ prodromal AD (PR AD; $F = 13$, $M = 15$; mean age = 70.8 years) patients, and $n = 13$ patients at the dementia stage of AD (AD dementia; $F = 8$, $M = 5$; mean age = 75.2 years). B, Threshold scores in the same cohort. C, Discrimination scores in the same cohort. D, Identification scores in the same cohort. E, Identification scores in $n = 29$ A- CN and $n = 15$ A+ CN participants subclassified into $n = 8$ A+ T- (purple) and $n = 7$ A+ T+ (pink). A-E, Mean $\pm$ standard error of the mean, Kruskal-Wallis and ordinary one-way analysis of variance statistical tests. F, Linear regression analysis between olfactory threshold scores and the ratio of OMP+P-tau181+ to OMP+tau+ cells established in Figure 4B on nasal smears. A, amyloid; AD, Alzheimer's disease; CN, cognitively normal; OMP, olfactory marker protein; p-tau, phosphorylated tau; T, tau; TDI, threshold/discrimination/identification.

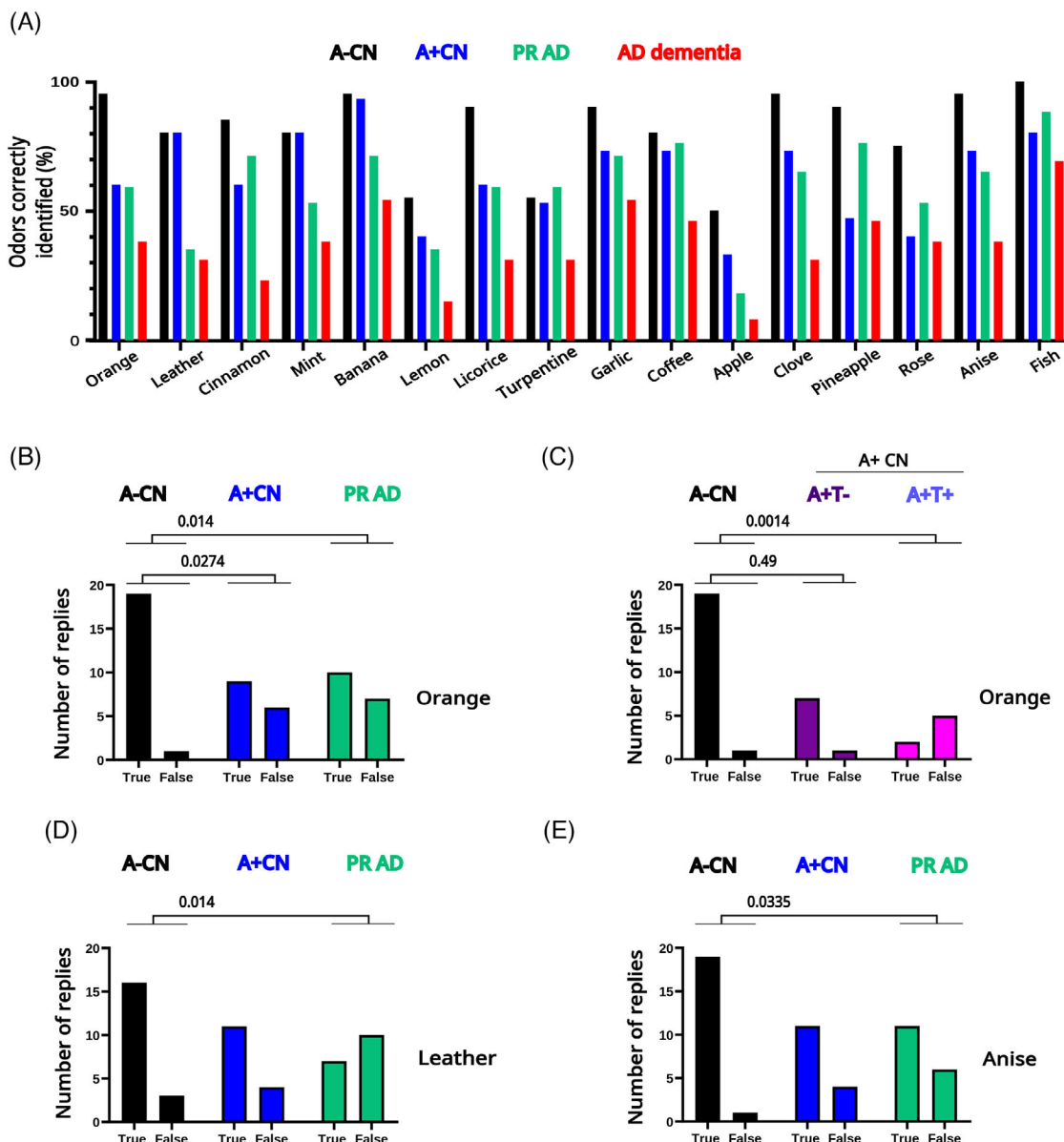


FIGURE 6 Differential impairment of odor identification across AD stages. A, Percentage of odors correctly identified by the participants for the 16 odors in the Sniffin' Sticks test was calculated as a percentage of total true responses in $n = 20$ A- CN (black) and $n = 15$ A+ CN participants (blue), $n = 17$ prodromal AD (PR AD) patients (green), and $n = 13$ patients at the dementia stage of AD (dementia AD; red). B, Number of replies for the orange scent in $n = 20$ A- CN (black), $n = 15$ A+ CN (blue), and $n = 17$ PR AD (green). C, Number of replies for the orange scent in $n = 20$ A- CN (black), $n = 8$ A+T- CN (purple), and $n = 6$ A+T+ CN (pink) participants. True: correct answer; False: wrong answer. D, E, Same as (B) for the indicated scents. B-E, Fisher exact tests. A, amyloid; AD, Alzheimer's disease; CN, cognitively normal; T, tau.

on the most informative odors within the A+CN group, we applied predefined selection criteria: (1) odors were required to have a correct identification rate of at least 80% in the A- CN group, thereby excluding odors with low baseline performance in CN individuals; (2) identification performance in the A+ CN group had to show a reduction of at least 30% compared to the A- CN group; and (3) this reduction had to be consistent across the AD continuum. Based on these criteria, identification of the orange scent was significantly impaired from the A+ CN preclinical stage ($p = 0.0274$; Figure 6B). We further compared by stratifying the A+CN group and observed that the significant alteration in orange identification was actually driven by

A+T+ patients ($p = 0.0014$; Figure 6C). Following the same criteria, identification of leather and anise scents showed a delayed decline, with impairments appearing only from the prodromal stage onward (Figure 6D-E).

Collectively, the presence of selective odor identification deficits in the preclinical phase without accompanying threshold impairments suggests that the olfactory system is more likely affected downstream in AD pathogenesis, rather than serving as an initial trigger. Notably, the early deficit in orange odor identification, limited to T+ individuals within the CN group, further supports the involvement of tau pathology in this downstream effect.

4 | DISCUSSION

4.1 | Overview

The reference model of tau propagation in AD, based on Braak staging, describes a “prion-like,” trans-synaptic spread from central to peripheral cortical regions.^{12,54} As olfactory deficits often precede cognitive symptoms, some authors have proposed an ascending prion-like propagation model initiated in peripheral structures such as the ONE and progressing toward the OB and cortex.^{16,55,56} This model is supported by the vulnerability of the ONE, which is directly exposed to environmental agents and connected to the OB without blood–brain barrier protection.^{22,52,57} However, this hypothesis has been challenged by studies reporting a central-to-peripheral trajectory, with tau emerging in medial temporal and entorhinal regions before involving olfactory cortices and peripheral structures.^{32,58,59}

Thus, the actual dynamics of tau pathology in the olfactory system remain controversial, notably because most available data derive from *post mortem* tissues at advanced stages, making it difficult to infer the true chronology of spread.^{26,27} In this study, we combined cellular, molecular, and functional approaches to explore whether the olfactory system plays a primary or secondary role in the etiology of AD.

4.2 | Propagation of tau pathology in the olfactory system

We demonstrate that in AD, NFT accumulation initially appears in the AON layer and extends to the ONL only at more advanced Braak stages (V–VI). In parallel, no tangles were detected in neuronal cell bodies of the ONE, either in nasal smears or in two *post mortem* ONE samples (Braak stage II). Nevertheless, nasal smears from patients at the dementia stage showed elevated p-tau181 levels. These findings may appear to contrast with previous reports describing tau pathology in the ONE, including tau-positive neuronal cell bodies.^{26,27} However, those observations are largely based on *post mortem* samples obtained at advanced stages of AD, and to date no study has demonstrated the presence of tau aggregates in the ONE at very early stages of the disease. Moreover, when tau pathology is reported in the ONE, aggregates are predominantly detected in supporting structures such as the lamina propria rather than within the neuroepithelial layer itself.³⁰ This suggests that nasal brushing, which does not collect such structures, may not be optimally suited for detecting pathological tau in the ONE. Future analyses of intact ONE tissues will be required to better characterize tau pathology across Braak stages.

Our findings on OB samples nevertheless align with previous reports indicating early NFT presence in the OB.^{17–20,60,61} The sequential involvement of OB layers supports a temporal spread pattern. Given that the olfactory cortex is affected at Braak stage I,¹⁰ the most consistent interpretation is a central-to-peripheral propagation toward the ONE via retrograde transport.⁶² However, it remains to be determined whether this progression reflects a prion-like mode of propagation or instead arises from differential cellular vulnerability across OB layers. Given that studies in transgenic mouse models such

as PS19 reported a propagation of tau from the periphery toward the central nervous system along the olfactory anterograde pathway,²⁴ it is also possible that, under specific experimental conditions, propagation may happen in both directions.

However, some limitations should be acknowledged in our study. The number of *post mortem* samples was limited, and the ONE tissue was not represented across all Braak stages. In addition, the heterogeneous cellular composition and low neuronal content of nasal smears hindered precise biochemical quantification of tau. Notably, the quantification of p-tau181 was performed on a limited number of nasal smear samples, which should be taken into account when interpreting the statistical significance of the observed associations. Addressing these limitations in future work will allow a more complete characterization of tau pathology along the olfactory pathway.

4.3 | Olfactory deficits as indicators of tau pathology propagation

Olfactory function was evaluated using the standardized Sniffin' Sticks test. Compared to healthy controls, our results indicate that odor identification scores have declined significantly already at the preclinical stage of AD. In contrast, discrimination scores were impaired at the prodromal stage, while threshold scores only declined at the dementia stage. Our results suggest that functional impairments are closely linked to the development of tau pathology within the anatomical regions responsible for the respective olfactory functions. The decline in identification scores, which involves mainly cognitive function, was observed in preclinical A+T+ individuals, with no difference observed between A–T– healthy controls and preclinical A+T–, reinforcing that olfactory loss is more strongly linked to tau pathology rather than to amyloid burden.^{63–65} This is consistent with previous reports showing that odor identification is typically the first olfactory function to be affected in the early stages of AD, a function primarily controlled by central olfactory regions.^{66,67} In particular, several studies have shown that loss of odor identification ability is associated with the presence of NFTs in the EC and the CA1 region of the hippocampus, both central olfactory structures affected from the earliest stages of the disease.^{11,68,69} Furthermore, we observed an emerging correlation between threshold decline and the p-tau181/total tau ratio in nasal brushing, a measure reflecting neuroepithelial activity, providing novel evidence that peripheral olfactory function might also be associated with tau pathology.

Importantly, the temporal sequence of olfactory impairments parallels the anatomical progression of tau pathology inferred from the conclusions drawn from the analysis of OB and nasal smears. Interestingly, discrimination function, partially dependent on the OB,^{70,71} is altered at the prodromal stage. This alteration appears to correlate with NFT-like aggregates detected in the bulb at the same stage in the immunostaining performed on the OB samples, in agreement with a previous report of discrimination impairment at similar stages.⁵⁸

Beyond global scores, the analysis of individual odorants revealed selective impairments. Identification of orange scent was already reduced at the preclinical stage for T+ participants and continued to

decline in prodromal and dementia stages. Leather and anise identification declined from the prodromal stage. This selective pattern, which confirms previous work for early affected orange scent in preclinical AD,³² supports a central origin of early olfactory deficits rather than a diffuse peripheral loss, which would be expected to affect identification of all odors. The specific early vulnerability of certain odorants, such as orange, raises questions about whether distinct neural circuits or receptor populations are selectively involved in the earliest phases of tau pathology spread.

From a translational perspective, the identification of specific odors and olfactory domains that are impaired at defined disease stages opens the possibility of developing targeted olfactory tests as complementary biomarkers for AD. In future work, longitudinal studies should investigate whether individuals with lower baseline olfactory performance, particularly in early-affected odorants, show faster clinical progression, as suggested by previous reports.^{15,52,72} Such approaches, combined with comparative studies in other major neurodegenerative disorders, will be essential to determine the specificity and predictive value of olfactory testing for AD.

4.4 | Limitations and future directions

Several considerations should be noted. *Post mortem* analyses of OB at Braak stage II were limited to a single case, and future studies including samples across the full range of Braak stages will be important to better capture disease progression. For a fraction of *post mortem* OB samples, the cause of death was unknown. For smear analyses, age-matched comparisons could not be systematically performed given the number of participants recruited, although the mean age difference across groups was relatively small. Another limitation is that the z scores used for cognitive stratification (CN, prodromal, dementia) were not adjusted for age, sex, or education. However, z scores were derived from a single, homogeneous reference sample of 32 amyloid-negative individuals who remained cognitively stable over an 8-year follow-up,⁴⁵ rather than from heterogeneous external normative datasets. This biologically and clinically well-characterized reference sample likely improved the consistency and comparability of standardized cognitive scores across domains and tests. In addition, cognitive stratification was reviewed by neurologists and neuropsychologists, incorporating functional status and all available clinical information. Finally, analyses in the A+ CN group were conducted in a relatively small cohort ($n = 15$, 8 T− and 7 T+), which may reduce statistical power; nevertheless, these findings provide valuable preliminary observations that warrant replication in larger samples.

Finally, although patients with Lewy bodies disorders were excluded as rigorously as possible based on clinical criteria, the lack of sensitive in vivo diagnostic tools means that the presence of undetected Lewy body pathology cannot be entirely ruled out. Given its known impact on olfactory function,⁷³ this potential overlap may represent a limitation, as part of the observed olfactory deficits might not be exclusively attributable to AD pathology.

Overall, our results highlight the strong potential of olfactory testing for improving AD diagnosis and staging. Expanding odor panels linked to early misidentification may enhance diagnostic value. However, the absence of detectable tau aggregates in nasal smears from pre-clinical individuals suggests limited utility for early diagnosis. Future proteomic and transcriptomic analyses may help identify alternative early biomarkers in the ONE. Additionally, possible contributions of anterograde olfactory pathways to early OB involvement warrant further investigation, opening new perspectives for AD research and prevention.

ACKNOWLEDGMENTS

We sincerely thank all the participants who generously volunteered for nasal brushing and olfactory testing. We thank members of Anabelle Decottignies, Bernard Hanseeuw, and Pascal Kienlen-Campard's laboratories for helpful comments and suggestions. The authors also thank the Netherland Brain Bank for providing the brain samples that have been used in this study and all the research participants who provided time and health data to make this research possible. Kamar Bouchoucha and Lisa Collin performed the experimental work, supervised by Anabelle Decottignies. Kamar Bouchoucha, Bernard Hanseeuw, Emilien Boyer, and Lise Colmant recruited the patients. Bernard Hanseeuw, Lara Huyghe, Lisa Quenan, Thomas Gerard, and Yasmine Salman contributed to the diagnosis, supervised by Bernard Hanseeuw. Kamar Bouchoucha, Lisa Collin, and Céline Coquette performed the olfactory tests, supervised by Caroline Huart. Caroline Huart and Valérie Hox performed the nasal brushings. Kamar Bouchoucha, Anabelle Decottignies, Bernard Hanseeuw, and Caroline Huart analyzed the data and wrote the paper. Pascal Kienlen-Campard critically revised the manuscript and contributed to writing. This work was supported by a grant from UCLouvain (Action de Recherches Concertées) to P.K.C., B.H., A.D., and C.H. K.B. was supported by UCLouvain (Action de Recherches Concertées). A.D. is supported by the Belgian Fund for Scientific Research (FNRS). C.C. is supported by the Télévie/FNRS. B.H. is supported by the FNRS (CCL40010417), the FRFS-WELBIO (#40010035), and the StopAlzheimer Foundation. P.K.C. is supported by a grant from the Fondation Louvain.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest. Author disclosures are available in the [supporting information](#).

ETHICS STATEMENT

All participants provided written informed consent prior to inclusion in the study.

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How to cite this article: Bouchoucha K, Collin L, Coquette C, et al. Olfactory decline tracks central-to-peripheral spread of tau pathology in Alzheimer's disease. *Alzheimer's Dement.* 2026;22:e71402. <https://doi.org/10.1002/alz.71402>