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Featured Article

Profound degeneration of wake-promoting neurons in Alzheimer's disease

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Abstract

Introduction: Sleep-wake disturbances are a common and early feature in Alzheimer's disease (AD). The impact of early tau pathology in wake-promoting neurons (WPNs) remains unclear.
Methods: We performed stereology in postmortem brains from AD individuals and healthy controls to identify quantitative differences in morphological metrics in WPNs. Progressive supranuclear palsy (PSP) and corticobasal degeneration were included as disease-specific controls.
Results: The three nuclei studied accumulate considerable amounts of tau inclusions and showed a decrease in neurotransmitter-synthetizing neurons in AD, PSP, and corticobasal degeneration. However, substantial neuronal loss was exclusively found in AD.
Discussion: WPNs are extremely vulnerable to AD but not to 4 repeat tauopathies. Considering that WPNs are involved early in AD, such degeneration should be included in the models explaining sleep-wake disturbances in AD and considered when designing a clinical intervention. Sparing of WPNs in PSP, a condition featuring hyperinsomnia, suggest that interventions to suppress the arousal system may benefit patients with PSP.

Keywords: Alzheimer's disease; Human; Tauopathies; Progressive supranuclear palsy; Corticobasal degeneration; Wake-promoting; Locus coeruleus; Orexin; Histamine; Sleep; Wakefulness; Unbiased stereology; Autopsy

1. Background

Sleep-wake disturbances are common in Alzheimer's disease (AD). Decreased sleep quality is associated with greater cognitive decline and lower quality of life, and it is one of the leading causes of institutionalization [1]. Sleep-wake

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disturbances occur early in the course of AD and involve an increase in nocturnal awakenings, a prominent decrease in slow-wave sleep, a modest decline in total sleep and rapid-eye-movement (REM) sleep time, and an increased propensity for daytime sleep [2–4]. Arousal deficiencies, such as excessive daytime sleepiness and sundowning, are significant complaints among patients with AD and may precede the onset of cognitive decline [5,6].

Arousal state requires the coordinated activity of various interconnected neurons. The classic sleep model postulates that wake-promoting neurons (WPNs) and sleep-promoting neurons (SPNs), both subcortically located, compete for network dominance through mutual inhibition, creating a systematic "switch" that results in either the sleep or awake state [7,8]. During wakefulness, WPNs exhibit high neuronal firing rates and suppress SPNs, whereas during sleep, WPNs are inhibited by SPNs [7,8]. WPNs include, among others, noradrenergic locus coeruleus (LC) neurons, orexin/hypocretin-producing neurons in the lateral hypothalamic area (LHA), and histaminergic neurons in the tuberomammillary nucleus (TMN). Many WPNs send excitatory projections to the cerebral cortex to stimulate cortical activation and behavioral arousal [9].

The neurological basis of arousal deficiencies in AD remains unclear. Animal and clinical imaging studies point to β-amyloid (Aβ) as the main contributor, particularly in disrupting slow-wave sleep [10]. Moreover, decreasing sleep escalates Aβ production and cortical deposition, as sleep normally promotes Aβ clearance [10].

Besides Aβ, which first accumulates in the cortex, a nonrandom accumulation of p-tau positive inclusions (AD-tau) constitutes AD's neuropathological hallmarks. AD-tau shows a stronger association with neuronal loss and clinical outcomes [11]. Nevertheless, little is known about the role of AD-tau in sleep-wake disturbances. Contrary to the Aβ pathology, AD-tau accumulates first in the brainstem and subcortical regions, later reaching allocortex and neocortex [12]. AD-tau inclusions often precede Aβ accumulation. We and others have shown that AD-tau inclusions in WPNs are among the first identifiable AD lesions in humans [13–15], which beg the question of whether a possible early and progressive degeneration of WPNs by AD-tau pathology contributes to arousal disturbances in AD. Unfortunately, the resolution achieved by tau-PET is still inadequate for enabling visualization of tau pathology in small subcortical nuclei *in vivo*, and systematic and quantitative neuropathological investigations of the arousal centers in AD remain elusive.

Here, we used design-based stereology in well-characterized postmortem brain tissues from patients with AD and healthy controls to quantitatively assess the differences in phospho-tau neuronal burden, neuronal loss, and neuronal ability to synthesize neurotransmitters in the wake-promoting network, focusing on the LC, LHA, and TMN. To further validate the clinical significance of our results in AD, we investigated the same brain areas in individuals with corticobasal degeneration (CBD) and progressive supranuclear palsy (PSP). CBD and PSP are tauopathies in which neurons from the arousal system develop tau inclusions, but unlike AD, they seldom present with arousal deficiencies [16,17]. In fact, patients with PSP experience hyperarousal [18]. Thus, we hypothesize that despite accumulating tau inclusions in arousal areas, WPNs will show smaller rates of neuronal and neurotransmitter loss in PSP and CBD than in AD.

2. Methods

2.1. Participants and neuropathological diagnosis

Disease subjects were selected based on a primary neuropathological diagnosis of AD, CBD, or PSP and absence of any other significant neurodegenerative or cerebrovascular changes. Normal control (NC) subjects were free of any cognitive impairment (Clinical Dementia Rating = 0), neurological or neuropathological diagnosis. Subjects with PSP, CBD, and NC scored as A $\leq$ 1B $\leq$ 1C0 according to the National Institute on Aging and Alzheimer's Association guidelines for the neuropathological assessment of AD [19]. Tissues were sourced from the Neurodegenerative Disease Brain Bank (NDBB) from the University of California, San Francisco (UCSF), and Brazilian BioBank for Aging Studies (BBAS) from the University of Sao Paulo [20] (Table 1). The NDBB receives brains from patients seen at the UCSF Memory and Aging Center. BBAS are population-based and host a high percentage of NC subjects who are not available in NDBB. Neuropathological assessments were performed using standardized protocols and followed the internationally accepted criteria for neurodegenerative diseases [21–23].

2.2. Tissue processing and immunohistochemistry

The specific protocol for tissue processing has been previously described (see [14] and Supplementary Material). Basically, celloidin-embedded brainstem and diencephalon blocks were cut in coronal or horizontal serial sections. Section orientation does not affect the optical fractionator probe in stereology. LC neurons were quantified in 60-μm-thick sections double-immunostained for tyrosine hydroxylase (TH) and phospho-Ser202 tau (CP13) at 1200-μm intervals. LHA (or TMN) was quantified in 30-μm-thick sections double-immunostained for orexin A (or histidine decarboxylase; HDC) and CP13 at 300-μm intervals. Sections were counterstained using gallocyanin stain (Fig. 1).

2.3. Stereological quantification

Stereologically-determined estimates were made for the (1) neurotransmitter-producing (i.e., TH+, orexin+, or HDC+) neuronal population, (2) p-tau+ neuronal population, and (3) total neuronal population (Table 2;

Table 1
Demographics stratified by diagnostic group and grouped by nucleus

Characteristics	Controls (n = 7)	AD (n = 13)	PSP (n = 7)	CBD (n = 7)	*P* value
Locus coeruleus					
N	6	8	5	4	
Age, mean (SD), y	61 (10.56)	66.12 (8.95)	68 (5.52)	69 (6.88)	.429
Males, No. (%)*	5 (83.33)	6 (75)	4 (80)	3 (75)	1
Education, mean (SD), y†	8.33 (3.39)	16.38 (2.39)	17.33 (1.15)	18 (2.16)	.005
CDR-SOB, median (IQR)	0 (0)	3 (0.25)	0.5 (0)	1.75 (2.5)	.001
Brain weight, mean (SD), g‡	1305 (132)	1122 (105)	1322 (175)	1177 (218)	.053
PMI, mean (SD), h	16 (4)	10 (5)	15 (13)	11 (8)	.22
Lateral hypothalamic area					
N	5	12	5	6	
Age, mean (SD), y	59.4 (11.1)	64.75 (7.71)	66.4 (6.88)	66.5 (6.66)	.396
Males, no. (%)*	3 (60)	9 (75)	4 (80)	4 (66.67)	.94
Education, mean (SD), y†	9.2 (4.76)	16.92 (2.39)	16.5 (1.91)	16.2 (3.49)	.019
CDR-SOB, median (IQR)§	0 (0)	3 (1.5)	0.5 (0)	0.5 (0)	.002
Brain weight, mean (SD), g‡	1236 (82)	1108 (109)	1303 (172)	1177 (144)	.051
PMI, mean (SD), h	14 (3)	12 (9)	13 (14)	10 (6)	.321
Tuberomammillary nucleus					
N	5	6	5	5	
Age, mean (SD), y	57.8 (8.58)	65.83 (9.99)	65.8 (7.05)	66.8 (7.4)	.332
Males, no. (%)*	3 (60)	4 (66.67)	5 (100)	3 (60)	.581
Education, mean (SD), y†	10.4 (2.88)	16.5 (2.81)	16 (2)	15 (2.58)	.042
CDR-SOB, median (IQR)§	0 (0)	2.5 (1.75)	0.5 (0.5)	0.5 (0)	.002
Brain weight, mean (SD), g‡	1270 (103)	1120 (131)	1350 (142)	1177 (144)	.055
PMI, mean (SD), h	15 (3)	13 (4)	7 (2)	7 (2)	.007

NOTE. *P* values were calculated by a Kruskal-Wallis rank-sum test comparing across the diagnostic groups unless otherwise stated. Controls were sourced from BBAS, and AD, CBD, and PSP from UCSF NDBB.

Abbreviations: CDR-SOB, Clinical Dementia Rating Sum of Boxes score; PMI, postmortem interval; AD, Alzheimer's disease; CBD, corticobasal degeneration; PSP, progressive supranuclear palsy; SD, standard deviation; UCSF NDBB, University of California, San Francisco, Neurodegenerative Disease Brain Bank; ICR, interquartile range.

**P* value for sex composition computed by Fisher's exact test.

†Data on education missing for two cases examined for the locus coeruleus, two cases for the lateral hypothalamus, and three cases for the tuberomammillary nucleus.

‡Brain mass missing for one case examined for the locus coeruleus, two cases for the lateral hypothalamus, and one case for the tuberomammillary nucleus.

§CDR-SOB missing for two cases examined for the lateral hypothalamus and one case for the tuberomammillary nucleus.

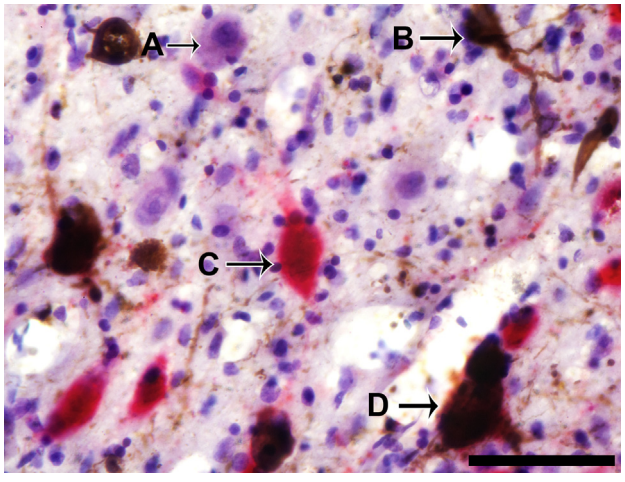

Fig. 1. Neuronal classification using double staining immunohistochemistry. The figure depicts an example of how the neurons were classified: (A) a neuron negative for p-tau and not expressing the neurotransmitter of interest (shown in purple); (B) a neuron positive for phospho-tau only (in brown); (C) a neuron positive for neurotransmitter only (in this case, orexin; in red); (D) a neuron positive for both phospho-tau and neurotransmitter of interest (orexin). This photo was taken from the lateral hypothalamic area from a case with Alzheimer's disease. The black bar represents 100 μm.

Supplementary Table). LC was identified using Olszewski's "Cytoarchitecture of the Human Brain Stem" [24] and was confirmed with TH staining. LHA and TMN were identified using online Allen Brain Atlas (Allen Institute for Brain Science) and were confirmed with orexin and HDC staining, respectively. Astroglial p-tau inclusions are enriched in cortical areas and rather scarce in subcortical nuclei in CBD and PSP [25]; thus, we focused on neurons. The specific protocol for optical fractionator probe has been previously described [14,26]. The coefficient of repeatability derived from the Bland-Altman plot showed consistency in counts between two trained, blinded investigators.

2.4. Statistical analyses

Pairwise differences in stereological estimates were assessed between disease groups for each nucleus as estimated size for each measured population and proportions of each subpopulation (e.g., TH+). The proportion of neurons positive for different subpopulations was determined by dividing the number of neurons positive for a given marker (estimates

Table 2
Mean (SD) stereological estimates for different characteristics of the LC, LHA, and TMN, stratified by neuropathologic group

Characteristics	Controls	AD	PSP	CBD
Locus coeruleus				
Neuron population, mean (SD)	77,574 (6121)	19,516 (9700)	36,186 (12,644)	38,491 (21,542)
p-Tau + neuron population, mean (SD)	3082 (3031)	7006 (4038)	27,271 (6676)	22,076 (18,531)
TH + neuron population, mean (SD)	74,636 (4013)	14,031 (7874)	27,747 (12,193)	36,315 (25,370)
Percentage of neurons with p-Tau, mean (SD), %	4 (3)	36 (16)	77 (11)	61 (50)
Percentage of neurons with TH, mean (SD), %	96 (4)	70 (12)	76 (14)	88 (45)
Lateral hypothalamic area				
Neuron population, mean (SD)	71,768 (35,107)	20,194 (16,656)	57,260 (25,907)	59,529 (43,447)
p-Tau + neuron population, mean (SD)	142 (203)	8174 (8287)	29,887 (14,182)	23,303 (12,708)
Orexin + neuron population, mean (SD)	68,576 (32,625)	14,000 (11,619)	30,677 (15,785)	38,886 (30,990)
Percentage of neurons with p-Tau, mean (SD), %	0 (0)	40 (15)	51 (15)	45 (12)
Percentage of neurons with orexin, mean (SD), %	97 (6)	68 (13)	56 (22)	63 (11)
Tuberomammillary nucleus				
Neuron population, mean (SD)	154088 (35,500)	58,468 (18,604)	125831 (20,378)	136469 (47,036)
p-Tau + neuron population, mean (SD)	477 (682)	14,523 (10,095)	13,004 (4603)	24,932 (26,878)
HDC + neuron population, mean (SD)	152141 (35,829)	48,218 (24,672)	116333 (16,429)	117467 (45,656)
Percentage of neurons with p-Tau, mean (SD), %	0 (0)	30 (23)	10 (3)	18 (18)
Percentage of neurons with HDC, mean (SD), %	99 (1)	79 (18)	93 (3)	87 (14)

Abbreviations: AD, Alzheimer's disease; PSP, progressive supranuclear palsy; CBD, corticobasal degeneration; TH, tyrosine hydroxylase; HDC, histidine decarboxylase; LC, locus coeruleus; LHA, lateral hypothalamic area; TMN, tuberomammillary nucleus; SD, standard deviation.

1 or 2) by the total neuron population (estimate 3), represented as a percent. Pairwise differences were analyzed using the Wilcoxon signed-rank test, with the alpha level set at 0.05. All analyses were conducted in the statistical computing program R (version 3.4.4; R Foundation for Statistical Computing, Vienna, Austria).

3. Results

Table 1 depicts demographics for the 34 cases in this study stratified by neuropathological diagnosis. Overall, the participants had an average (standard deviation) age of 65.2 (7.9) years, and 76.5% were males. There were no differences between the groups regarding age and proportion of males (Table 1). In some cases, portions of the hypothalamus and brainstem had already been sampled for other studies and thus could not be included in the analysis because the unbiased stereological method requires the availability of the whole region of interest (Table 1). Table 2 gives absolute and relative numbers of the total neuronal population, neurons synthesizing neurotransmitter, and neurons bearing p-tau inclusions. Table 3

Table 3
P values from a Wilcoxon rank-sum test comparing the different stereological estimates between different pairs of the neuropathologic groups

Characteristics	Controls vs. AD	Controls vs. PSP	Controls vs. CBD	AD vs. PSP	AD vs. CBD	PSP vs. CBD
Locus coeruleus						
Neuron population	0.001	0.004	0.010	0.030	0.073	0.730
p-Tau + neuron population	0.081	0.004	0.019	0.002	0.073	0.286
TH + neuron population	0.001	0.004	0.010	0.045	0.214	0.730
Percentage of neurons with p-Tau	0.001	0.004	0.010	0.002	0.683	0.286
Percentage of neurons with TH	0.001	0.009	0.352	0.435	0.570	0.905
Lateral hypothalamic area						
Neuron population	0.002	0.548	0.792	0.009	0.024	0.999
p-Tau + neuron population	0.002	0.011	0.008	0.009	0.005	0.931
Orexin + neuron population	0.001	0.056	0.247	0.014	0.067	0.999
Percentage of neurons with p-Tau	0.002	0.011	0.008	0.104	0.553	0.537
Percentage of neurons with orexin	<0.001	0.016	0.004	0.195	0.180	0.429
Tuberomammillary nucleus						
Neuron population	0.004	0.094	0.690	0.008	0.017	0.834
p-Tau + neuron population	0.004	0.012	0.008	0.927	0.537	0.834
HDC + neuron population	0.004	0.094	0.310	0.008	0.017	0.834
Percentage of neurons with p-Tau	0.004	0.012	0.008	0.410	0.537	0.402
Percentage of neurons with HDC	0.030	0.012	0.016	0.410	0.662	0.834

Abbreviations: AD, Alzheimer's disease; PSP, progressive supranuclear palsy; CBD, corticobasal degeneration; TH, tyrosine hydroxylase; HDC, histidine decarboxylase.

depicts pairwise comparisons between disease groups for all tested parameters.

3.1. Noradrenergic LC

3.1.1. The LC exhibits a profound neuronal loss in AD, whereas neuronal loss is milder in CBD and PSP

Compared with NC, we detected, 74.84% ($P = .001$) fewer LC neurons in AD, 50.38% ($P = .010$) fewer in CBD, and 53.35% ($P = .004$) fewer in PSP (Table 1). Within tauopathies, AD exhibited significantly fewer LC neurons than PSP ($P = .030$) but not when compared with CBD ($P = .073$).

We found a similar pattern of loss when investigating the percentage of surviving neurons able to synthesize norepinephrine (measured by proxy using TH). The mean (±standard deviation) percent of LC neurons with TH positivity in NC was 96% ± 4%. In AD, this percentage dropped to 70% ± 12%, whereas in PSP and CBD, the percentages dropped to 76% ± 14% and 88% ± 45%, respectively. These results indicate that in AD, not only neuronal loss in the LC is severe but a significant percentage of surviving neurons show signs of impaired norepinephrine synthesis. Interestingly, PSP also showed impaired norepinephrine synthesis despite having a milder neuronal loss than in AD (Figs. 2 and 3A).

3.1.2. The percentage of surviving neurons containing p-tau inclusions is higher in 4R tauopathies than in AD

Although our NC subjects had p-tau inclusions in 4% ± 3% of neurons, these numbers grew significantly to 36% ± 16% in AD, 77% ± 11% in PSP, 61% ± 50% in CBD. Interestingly, the percentage of surviving p-tau + neurons was significantly lower in AD than in PSP ($P = .002$).

3.2. Orexinergic LHA—the neuronal loss is significant in AD but not in PSP or CBD

Similar to LC, the neurons in LHA showed a severe 71.86% average loss in AD ($P = .002$) (Table 2). However, 97% ± 6% of neurons synthesize orexin in NC subjects, and this percentage fell to 68% ± 13% ($P < .001$) in AD.

Similar to AD, orexinergic neurons accumulate abnormal tau (51% ± 15% and 45% ± 12% of neurons in PSP and CBD, respectively) (Figs. 2 and 3B). Also, the percentage of neurons synthetizing orexin diminished in PSP (56% ± 22%; $P = .016$) and CBD (63% ± 11%; $P = .004$), but as opposed to AD, the total number of neurons remained similar to that of NC subjects and significantly higher than that in AD (PSP vs. AD: $P = .009$ and CBD vs. AD: $P = .024$).

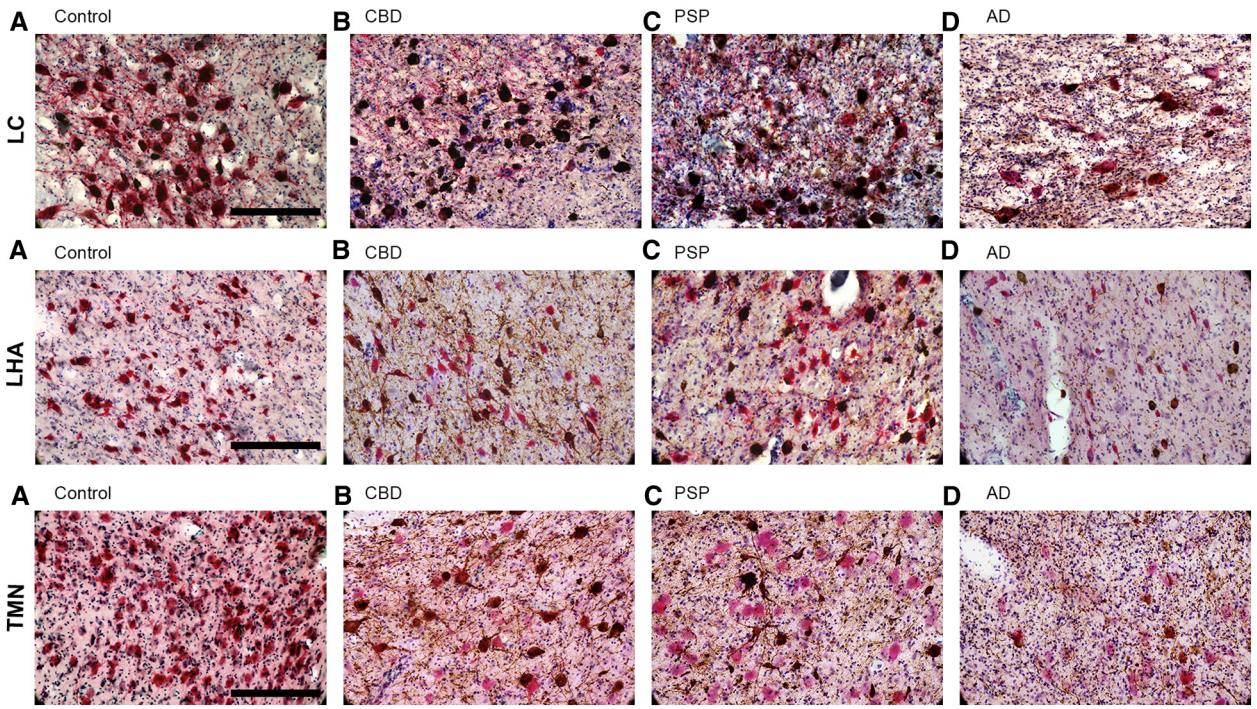

Fig. 2. Representative histological images of wake-promoting nuclei. LC (top), LHA (middle), and TMN (bottom) tissue sections are depicted with their corresponding neurotransmitter/enzymes (TH, orexin, or HDC, respectively; in red) and tau (CP13; in brown). Notice smaller neuronal population in AD in all three wake-promoting nuclei. Some LC neurons contain neuromelanin and appear pigmented. The black bar represents 200 μm. From left to right column: (A) control, (B) CBD, (C) PSP, (D) AD (20× magnification). Abbreviations: LC, Locus coeruleus; LHA, lateral hypothalamic area; TMN, tuberomammillary nucleus; TH, tyrosine hydroxylase; HDC, histidine decarboxylase; AD, Alzheimer's disease; CBD, corticobasal degeneration; PSP, progressive supranuclear palsy.

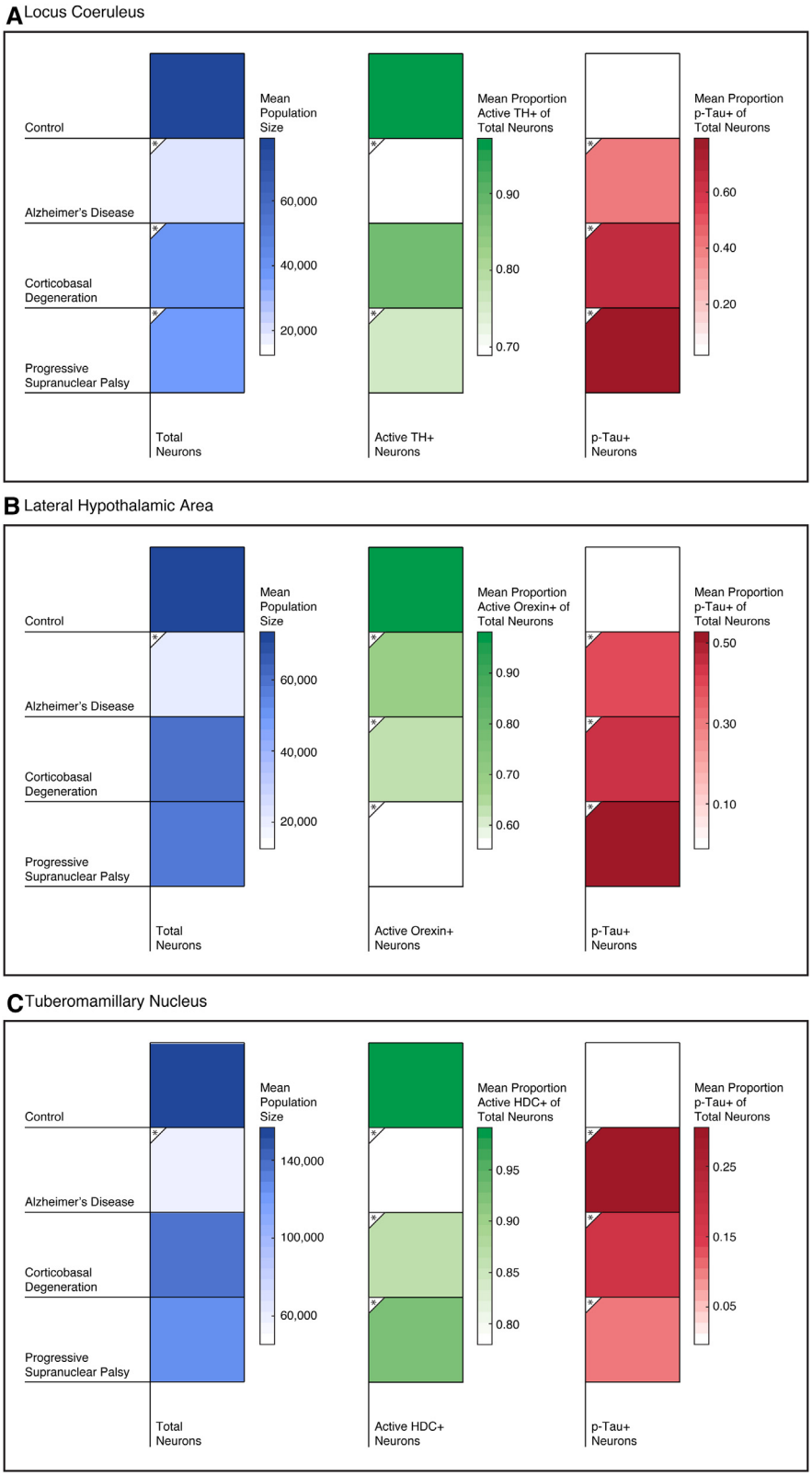

Fig. 3. Stereological comparison of wake-promoting nuclei in tauopathies. The arousal network is extremely vulnerable to AD-tau toxicity as shown by the severe neuronal loss in (A) LC, (B) LHA, and (C) TMN in cases of AD, whereas the same network is relatively spared in cases of PSP and CBD. The blue column depicts mean population size, the green column depicts the mean proportion of neurotransmitter + neurons, and the red column depicts the mean proportion of p-tau + neurons. Scale bars are shown to the right. *Denotes statistical significance in comparison to the control group. Abbreviations: LC, locus coeruleus; LHA, lateral hypothalamic area; TMN, tuberomammillary nucleus; TH, tyrosine hydroxylase; HDC, histidine decarboxylase; AD, Alzheimer's disease; CBD, corticobasal degeneration; PSP, progressive supranuclear palsy.

3.3. Histaminergic TMN—similar to LHA, TMN neuronal loss is specific to AD

In NC subjects, almost all the TMN neurons were HDC positive (99% ± 1%). We detected, on average, a 62.06% ($P = .004$) reduction in the total number of neurons in AD (Table 1). Conversely, the total number of neurons in CBD and PSP remained similar to that of NC subjects and significantly higher than that in AD (PSP vs. AD: $P = .008$; CBD vs. AD: $P = .017$). In all three tauopathies, the percentage of remaining neurons containing HDC and p-tau was significantly lower and higher, respectively, than that in NC subjects (Tables 2 and 3; Figs. 2 and 3C).

4. Discussion

In this quantitative postmortem study, we used design-based stereology to investigate morphological changes associated with tauopathies in three of the main brain nuclei containing WPNs—the LC, LHA, and TMN—to demonstrate that the arousal network is extremely vulnerable to AD-tau toxicity as shown by the severe neuronal loss, whereas the same network is relatively spared in PSP and CBD, even at late disease stages. Although all three nuclei substantially accumulate p-tau inclusions in AD, PSP, and CBD, we found greater than 60% neuronal loss of LC, LHA, and TMN neurons in AD, whereas the neuronal loss in CBD and PSP was significantly mild in LC and undetectable in LHA and TMN. Nevertheless, despite being spared of neuronal loss, we detected a reduced percentage of neurons synthesizing their corresponding neurotransmitter (TH+, orexin+, or HDC+) in PSP and CBD, as we did in AD.

AD and PSP feature clear sleep-wake disturbances of different nature. While patients with AD display increased sleep fragmentation with relatively preserved total sleep time and arousal deficiencies such as daytime napping and sundowning (late-day confusion) [27], patients with PSP feature decreased non-rapid eye movement and REM sleep, elevated gamma activity in both wake and sleep electroencephalogram (hyperarousal), and prolonged sleep latencies on multiple sleep latency tests, meaning that individuals with PSP have much less total sleep time [18]. In CBD, sleep-wake disturbances have been suggested in clinical studies, but the features are unclear mainly because only 1 of 3 patients with the corticobasal syndrome, the most common clinical manifestation of CBD, has an underlying CBD pathology.

Recent studies have been focusing on the mechanisms underlying sleep-wake disruption in AD. Experimental studies have shown that the accumulation of cortical Aβ can cause sleep disruption and, in a positive feedback loop, further exacerbate Aβ deposition because Aβ is best metabolized during sleep [28]. Similar dynamics have been observed in human studies using cerebrospinal-fluid (CSF) analysis and neuroimaging [28]. Individuals with Aβ deposition had a poorer sleep quality than those without Aβ deposition [29,30], and cortical Aβ burden correlates with impaired generation of non-REM slow-wave oscillation [31]. A recent study by Holth et al. showed that levels of interstitial fluid tau fluctuate between sleep and wake states in mice and that sleep deprivation increases levels of mice interstitial fluid tau and human CSF tau [32], suggesting a possible role of AD-tau in modulating sleep disturbances, such as sleep fragmentation. In summary, most models of sleep-wake disturbances in AD focus on sleep disturbances and consider arousal deficiencies as a secondary, compensatory consequence of sleep impairment. Very little data are available to examine the possibility that AD-related degeneration of the arousal system directly contributes to arousal deficiencies in AD.

Different neuronal populations modulate sleep (SPNs) and wake control (WPNs) and mutually inhibit each other's activity, resulting in a behavioral wake or sleep state [9]. WPNs are distributed in specific nuclei in the brainstem, hypothalamus, and basal forebrain and discharged in an orchestrated manner to promote cortical arousal (for a comprehensive review, see the study by Brown et al. [9]). We focused on noradrenergic, orexinergic, and histaminergic WPNs because they are highly interconnected and play an integral part in governing consolidated wakefulness and sleep-to-wake transition.

The noradrenergic LC sends dense excitatory projections to extensive brain areas and inhibitory projections to SPNs in the intermediate nucleus of the hypothalamus (analog of the ventrolateral preoptic nucleus in rodents) [9]. LC neurons exhibit the highest firing rate during waking and silence during non-rapid eye movement or REM sleep in animal models [33]. LC neurons are among the first to accumulate p-tau in AD and suffer a catastrophic neuronal loss along AD progression [13,14]. The LC-norepinephrine system becomes highly dysfunctional in AD pathology. Here, we detected a milder LC neuronal loss in PSP and CBD than in AD, confirming our previous results from semiquantitative methods [16]. These findings either represent the fact that LC involvement in PSP and CBD may occur later in the disease progression than in AD or that CBD-tau and PSP-tau are less toxic to LC neurons than AD-tau. Nonetheless, both hypotheses reflect a relatively simplistic model in which tau neurotoxicity is a product of a time of exposure and/or "toxicity of a strain" with little influence of intrinsic neuronal factors. Interestingly, the percentage of surviving p-tau + neurons was significantly higher in PSP than in AD ($P = .002$). Considering the more severe LC neuronal loss in AD, this finding may be seen paradoxical, begging the question of whether intrinsic factors make LC neurons more resistant to PSP-tau. Curiously, we showed previously that PSP-tau correlates with a more severe neuronal loss in substantia nigra than AD-tau [16], despite the fact that SN also accumulates AD-tau from Braak stage 0 [15]. Studies including cases at

progressive PSP and CBD stages might help to clarify this question and answer whether the surviving neurons seen in the LC in AD may represent the subpopulation resilient to p-tau. In addition, LC neuronal loss has been previously shown to be topographically arranged in AD [34–36]. For instance, some studies indicate that heaviest neuronal loss occurs in the central area of LC (projecting to temporal cortex/hippocampus), whereas neurons in the rostral and caudal areas of LC (projecting mainly to frontal/occipital regions) are relatively spared [35]. Given these topographical characteristics in AD, future studies examining whether CBD and PSP show a similar pattern of neuronal loss in LC will further inform us of the heterogeneous nature of the noradrenergic system. Regardless, our results highlight the need to quantify multiple parameters to understand the impact of a neurodegenerative condition. A stand-alone measurement of tau (or any other misfolded protein) burden is a poor indicator of the neurodegeneration status.

Orexinergic neurons in the LHA play an important role in wakefulness. Orexin knockout mice, orexin-receptor knockout mice, and orexinergic neurons–ablated transgenic mice all show a pattern resembling human narcolepsy [37]. Orexinergic neurons accumulate AD-tau from Braak stage 0 [15], but the impact of AD on the orexinergic system remains unclear. Most studies measured orexin CSF levels and show contradicting results. Schmidt et al. showed an unaltered CSF orexin level in patients with moderate to severe AD compared with that in healthy controls [38], whereas Liguori et al. reported increases in CSF orexin levels in AD featuring moderate to severe cognitive decline [39]. In a previous postmortem study examining orexinergic neurons in AD, Fronczek et al. reported a 40% decrease in neuronal number in late-stage AD, using semiquantitative methods [40]. Here, using unbiased stereology, we report an even greater neuronal loss in AD with 71.85% reduction. To put this into another perspective, patients with narcolepsy (a chronic sleep disorder that causes overwhelming daytime drowsiness) have been reported to show 85-95% reduction in the number of orexinergic neurons [41], almost comparable to what we see in patients with AD. While direct comparisons should be examined with caution because of differences in the methodologies used, this highlights an abnormally high magnitude of orexinergic loss in AD.

Little is known about LHA neurons in 4R tauopathies. Yasui et al. reported a significantly lower orexin CSF level in patients with probable CBD and PSP than that in those with Parkinson's disease [42] and speculated that such lower levels reflect a loss of orexinergic neurons or impaired orexin transmission. In our pioneering study investigating LHA neuronal numbers in PSP and CBD, we found a decrease in neuronal ability to synthetize orexin but failed to detect an orexinergic neuronal loss. Similar to the LC, LHA neurons accumulated CBD-tau and PSP-tau in large proportions, and future studies should explore the biological properties that confer resistance to death in orexinergic neurons in 4R tauopathies but not in AD, especially because these neurons are, in theory, rescuable. Regardless, an intriguing issue here is how to reconcile our findings of substantial LHA neuronal loss in AD, but not in CBD and PSP, with findings of increased CSF orexin levels in AD but decreased in PSP and CBD.

Histamine is a critical wake-promoting neurotransmitter. Almost all histaminergic neurons are located in the hypothalamic TMN. Previous neuropathological reports describe TMN vulnerability to AD-tau starting at Braak stage 0 with accompanying neuronal loss [15,43–45]. However, those studies were either qualitative, did not use histaminergic-specific neuronal markers, or only analyzed neurons at rostral TMN levels. An early study using single-label immunostaining found no significant differences in the number of histaminergic neurons between AD ($n = 3$) and cases with milder AD pathology lacking clinical decline [46]. That study preceded the Braak stage system; thus, it is unclear if results point to a lack of neuronal loss in TMN in AD or if losses occurred already in early AD stages. Interestingly, CSF studies have indicated either no change or only a modest decrease in CSF histamine/tele-methylhistamine level in AD clinical syndrome [47,48]. However, these studies could have been confounded by the fact that brain immune cells can synthesize histamine [49]. Here, we assessed the full extent of TMN using a histaminergic neuronal marker and demonstrate a significant 62% reduction in TMN neurons in late stages of AD. In CBD and PSP, although 18% and 10% of TMN neurons contained tau inclusions, respectively, TMN neuronal numbers were comparable to NC. But, similar to LHA neurons, in CBD and PSP (and also in AD), the proportion of histamine synthetizing neurons was smaller than that in controls, again suggesting that CBD-tau and PSP-tau have a degree of neurotoxicity to this neuronal subpopulation, which could be, theoretically, reversed.

In general, our results contrast with findings in noradrenergic, orexinergic, and histaminergic levels from CSF and in vivo studies. Some studies indicate that levels of CSF norepinephrine and orexin [39,50], TH-mRNA expression in LC neurons [51], and histamine release at axon terminals [52] are increased in advanced AD. In our study, however, the percentage of neurotransmitter-producing neurons was decreased in all tauopathies. Evidence suggests that a significant neuronal loss of neurons may lead to compensatory adaptations, including an increase in neurotransmitter expression per cell or turnover rate [53]. Our findings, especially regarding the massive neuronal loss in all three nuclei in AD, warrant for a reinterpretation of these CSF findings.

Our findings may inform on therapeutic strategies to improve sleeping quality in PSP. Patients with PSP experience profound sleep deprivation without recuperation,

suggesting a diminished homeostatic sleep drive [18]. As LHA and TMN are relatively preserved in PSP, it is possible that the lack of inhibition of WPN may underlie hyperarousal. Thus, strategies using orexin or histamine antagonists may help to restore WPN-SPN balance.

4.1. Limitation

Our study has a number of limitations. First, our sample size is relatively small because of the rarity of CBD and PSP cases, which is made even more difficult by our criterion to exclude cases with copathology and our requirement of whole hypothalamic and brainstem availability for the study. In addition, our AD cohort is relatively young (average age of death: 66 years), especially because we aimed to match the age of death across groups and excluded cases with mixed pathology. Although all included cases were sporadic, some evidence suggests that AD pathology in presenile and senile age groups may vary in certain aspects. Thus, our results may not be generalizable to a cohort of older participants with AD. Still, our study is one of the largest quantitative studies of its kind to date. Also, the disadvantage of our relatively small sample size is partially compensated by our in-depth clinical and postmortem characterization of the cases and the use of unbiased stereological methods. Second, our cohort has a greater proportion of male subjects, leading to potential sex-related bias. Although we and others failed to detect sex differences in LC [13,54], sex differences in LHA and TMN neuronal numbers have not been well addressed. Third, despite our effort to only include cases with short postmortem intervals, we cannot rule out a possibility of hypoxia-induced neuropathological changes, including the degradation of our proteins of interest. Fourth, LHA neurons are heterogeneous in nature, and thus, our counts may include some surrounding non-orexinergic neurons. However, we minimized this risk by accurately delineating LHA using cytoarchitecture, 3-dimensional reconstruction images, and orexin staining. Fifth, while all cases used in the study were Caucasians (in Brazilian cases, ethnicity is determined by DNA ancestry markers to avoid bias in such an admixed population) and underwent exactly same tissue processing, our disease cohort (UCSF NDBB) and control cohort (BBAS) were sourced from two different geographical regions. Sixth, while we conducted multiple tests without correcting for multiple comparisons, many of our tests were not truly independent. Nevertheless, this underscores the need for a larger sample and a limitation on the generalizability of these results. Another limitation is the unknown status of obstructive sleep apnea, a sleep disorder associated with hypoxia-reoxygenation that has been associated with dementia and may lead to daytime somnolence. Finally, our study is cross-sectional by nature and only includes cases that are at late stages, which makes it difficult to determine when pathological changes begin.

5. Conclusion

In conclusion, our study provides compelling evidence that WPNs are extremely vulnerable to AD-tau and relatively resistant to CBD-tau and PSP-tau. Our findings do not rebuke the bidirectional role of Aβ and sleep disturbances in AD but argue that AD-tau–driven degeneration of the arousal system should be included in the models explaining sleep-wake disturbances in AD and considered when designing a clinical intervention. A longitudinal, clinicopathological study combining objective sleep measurements with unbiased postmortem evaluation may further clarify the contribution of tau pathology to arousal deficiencies in AD. Also, our study corroborates the hypothesis that selective neuronal vulnerability to tauopathies at the cellular level is not a simple result of exposure time and "strain" toxicity. Wake-promoting nuclei accumulate PSP-tau and CBD-tau in a large proportion without proportional terminal neuronal loss. Investigating selective vulnerability to tau using nuclei with a relatively homogeneous neuronal population as a framework may inform on the intrinsic neuronal factors influencing vulnerability to tau toxicity.

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Supplementary Data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.jalz.2019.06.3916.

RESEARCH IN CONTEXT

1. Systematic Review: The authors thoroughly reviewed the literature using PubMed and cited appropriate articles. Only a small number of human studies have been conducted on the wake-promoting neurons in cases of Alzheimer's disease (AD), and systematic and quantitative neuropathological investigations of the arousal centers still remain elusive. Very little is known about wake-promoting neurons in 4 repeat tauopathies.
2. Interpretation: Our study provides compelling evidence that wake-promoting neurons are extremely vulnerable to AD-tau while relatively resistant to corticobasal degeneration–tau and progressive supranuclear palsy–tau. Furthermore, our study corroborates the hypothesis that selective neuronal vulnerability to tauopathies at the cellular level is not a simple result of exposure time and "strain" toxicity. For instance, unlike AD, wake-promoting nuclei accumulate progressive supranuclear palsy–tau and corticobasal degeneration–tau in a large proportion without proportional terminal neuronal loss.
3. Future Directions: Tau-driven degeneration of the arousal system should be included in the models explaining sleep-wake disturbances in patients with AD and considered when designing a clinical intervention.

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