Brain Research Bulletin 78 (2009) 97–104

Contents lists available at ScienceDirect

Brain Research Bulletin

journal homepage: www.elsevier.com/locate/brainresbull

Research report

Intralaminar nuclei of the thalamus in Lewy body diseases

Daniel Brooks, Glenda M. Halliday*

Prince of Wales Medical Research Institute and the University of New South Wales, Sydney, Australia

ARTICLE INFO

Article history:
Available online 18 September 2008

Keywords:
α-Synuclein pathology
Calretinin immunohistochemistry
Dementia with Lewy bodies
Intralaminar nuclei
Parkinson's disease
Thalamus

ABSTRACT

Although the intralaminar thalamus is a target of α-synuclein pathology in Parkinson's disease, the degree of neuronal loss in Lewy body diseases has not been assessed. We have used unbiased stereological techniques to quantify neuronal loss in intralaminar thalamic nuclei concentrating α-synuclein pathology (the anterodorsal, cucullar, parataenial, paraventricular, central medial, central lateral and centre-median/parafascicular complex) in different clinical forms of Lewy body disease (Parkinson's disease with and without dementia, and dementia with Lewy bodies, $N = 21$) compared with controls ($N = 5$). Associations were performed in the Lewy body cases between intralaminar cell loss and the main diagnostic clinical (parkinsonism, dementia, fluctuation in consciousness, and visual hallucinations) and pathological (Braak stage of Parkinson's disease) features of these diseases, as well as between cell loss and the scaled severity of the α-synuclein deposition within the intralaminar thalamus. As expected, significant α-synuclein accumulation occurred in the intralaminar thalamus in the cases with Lewy body disease. Pathology concentrated anteriorly and in the central lateral and paraventricular nuclei was related to the Braak stage of Parkinson's disease, ageing, and the presence of dementia. Across all types of Lewy body cases there was substantial atrophy and neuronal loss in the central lateral, cucullar and parataenial nuclei, and neuronal loss without atrophy in the centre-median/parafascicular complex. Cases with visual hallucinations showed a greater degree of atrophy of the cucullar nucleus, possibly due to amygdala denervation. The significant degeneration demonstrated in the intralaminar thalamus is likely to contribute to the movement and cognitive dysfunction observed in Lewy body disorders.

1. Introduction

Both Parkinson's disease (PD) and dementia with Lewy bodies (DLB) are characterized by the presence of α-synuclein immunopositive Lewy bodies in brainstem, basal forebrain and cortical (particularly limbic) regions [1,4,6,8,9,11,16,28]. The early clinical features of DLB are two of dementia, Parkinsonism, visual hallucinations and/or fluctuating cognition [28]. The early clinical features of PD are two of bradykinesia, rigidity, resting tremor and gait instability [11]. However, many PD cases progress over time (most commonly after 8 years) to also have visual hallucinations and fluctuating cognition which lead to dementia, or PD with dementia (PDD) [31]. The severity of Lewy pathology in PDD and DLB is often indistinguishable [13,24,26,27].

The thalamus is a key structure that integrates information flow between the regions concentrating Lewy pathology in these Lewy body diseases, and functional change has been identified in the thalamus during complex visual hallucinations similar to those described in Lewy body disease [2]. The intralaminar and midline nuclei, and the mediodorsal nucleus, have been implicated as important thalamic structures in arousal, vigilance and cognition [23,36,38]. Despite limited studies on thalamic pathology in Lewy body diseases, the thalamus similarly accumulates α-synuclein Lewy inclusions which concentrate in intralaminar regions [33] largely sparing the mediodorsal nucleus [19]. Of the limited regions examined, the caudal intralaminar nuclei of the thalamus, and not the mediodorsal or anterior nuclei, undergo substantial neuronal loss in PD [19], although their fate in PDD or DLB remain unknown. Neuronal loss in other intralaminar nuclei has not been studied. The aim of the current study is to remedy this lack of knowledge.

2. Methods

2.1. Cases

All participants were prospectively followed through enrolment in a regional brain donor program, as previously described [16]. Written consent for autopsy was obtained in all cases and the project was approved by the Human Ethics Committees of South Eastern Sydney Area Health Service and the University of New South Wales under the Human Tissue Act of the State of New South Wales. The average time between last clinical assessment and death was 17 ± 2.4 months. Clinical diagnoses were made by specialist neurologists and confirmed pathologically using standard research criteria [11,28]. Clinical features were documented by specialist neurol-

* Corresponding author. Tel.: +61 2 9399 1104.
E-mail address: g.halliday@powmri.edu.au (G.M. Halliday).

0361-9230/$ – see front matter © 2008 Elsevier Inc. All rights reserved.
doi:10.1016/j.brainresbull.2008.08.014

Table 1
Group demographics and pathological variables

	Control	PD	PDD	DLB
N (% male)	5 (40)	6 (83)	5 (60)	10 (90)
Age (year)	76 ± 6	64 ± 20	76 ± 5	72 ± 14
Postmortem delay (h)	20 ± 13	21 ± 10	16 ± 15	25 ± 17
Disease duration (year)	–	13 ± 4	11 ± 6	6 ± 3*
%Parkinsonian	0	100	100	100
%Demented	0	0	100	100
%Fluctuating consciousness	0	0	60	60
%Visual hallucinations	0	17	100	50
Braak PD stage (0–6)	0	4.3 ± 0.8	5.2 ± 0.4	5.6 ± 0.7
%Cortical plaque	0	17	40	90
Braak NFT stage (0–6)	0.75 ± 1.5	0.5 ± 0.8	2 ± 1.5	0.7 ± 0.7

* Significantly different from other Lewy body disease groups, posthoc protected *t*-test <0.05.

ogists when present. Visual hallucinations were carefully assessed and identified when they were complex and well-formed, as previously described [16]. Similarly, fluctuations in consciousness were recorded rather than fluctuations in cognition or periods of confusion, as previously described [16].

For the present study, all neurological and neurodegenerative diseases other than Lewy body syndromes were excluded, as were cases with head injury, brain tumour or obvious infarction. Twenty-one cases with Lewy body pathology were selected (Table 1): ten cases meeting current criteria for dementia with Lewy bodies (DLB [28]), five cases meeting criteria for idiopathic Parkinson's disease (PD [11]) who developed dementia years after symptom onset (PDD cases) and six non-demented PD only cases. Five age-matched controls without psychiatric abnormalities or diagnostic neuropathology were selected for comparison (Table 1). There were no differences in age at death ($F_{3,21} = 0.4$, $p = 0.8$) or postmortem delay ($F_{3,21} = 0.5$, $p = 0.7$) between diagnostic groups (Table 1). There were differences in gender number; however no differences were detected between genders for any clinical ($F_{3,21} < 0.5$, $p > 0.47$) or pathological measure ($F_{3,21} < 2.1$, $p > 0.17$).

2.2. Tissue preparation

The preparation of thalamic tissue for quantitation was the same as previously described [14,19,20]. Briefly, the thalamus, from the mammillothalamic tract to the beginning of the pulvinar, was excised from the formalin-fixed coronal brain slices prepared for diagnostic neuropathology. The 3 mm blocks of the thalamus were cryoprotected for 24 h in 30% sucrose in 0.1 M Tris–HCl buffer (pH 7.4) for 2 days, frozen to −20 °C and serially sectioned at 50 μm on a cryostat. Three series of sections spaced 750 μm apart were taken and stained using (i) cresyl violet (0.5%) and immunohistochemistry performed for (ii) α-synuclein (mouse antibody 610786, from BD Transduction Laboratories, 1:1000) and (iii) the calcium-binding protein calretinin (CalR, rabbit antibody 7699/4, from Swant, 1:20,000).

For the immunohistochemistry, the sections were processed free floating. Sections were placed in a solution of 3% hydrogen peroxide and 50% ethanol for 30 min at room temperature to quench endogenous peroxidases. The sections were washed briefly in distilled water to stop the reaction, and then rinsed twice in 0.1 M Tris buffer for 5 min each. To block non-specific binding sites, the slides were placed in a solution of 10% normal horse serum for 1 h at room temperature. The sections were then incubated overnight at 4 °C in primary antibody diluted in 1% normal horse serum. To stop the antigen/antibody reaction, they were rinsed in 0.1 M Tris for a total of 1 h. The sections were then incubated in the species-specific biotinylated secondary antibody (Vector Laboratories Inc., Burlingame, CA) diluted 1:200 in 1% normal horse serum at room temperature for 1 h and then rinsed in 0.1 M Tris for 30 min. The tertiary stage involved incubating the sections in an avidin–biotin–peroxidase tertiary complex (Vectastain, Vector Laboratories Inc., Burlingame, CA) diluted 1:500 in 1% normal horse serum at room temperature for 1 h. They were then again rinsed in 0.1 M Tris for 30 min. The antibody was visualised by incubating the sections with 0.7% hydrogen peroxide 0.15% diaminobenzidine tetrahydrochloride solution for 10 min at room temperature. After the reaction was stopped the sections were rinsed in distilled water, mounted onto gelatinised slides, air-dried overnight, then dehydrated through graded ethanol to xylene (70% ethanol, 95% ethanol, 2× 100% ethanol and 2× xylene for 5 min each) and coverslipped with DPX. The specificity of the immunohistochemical reaction was tested by omitting the primary antibody on test sections. No peroxidase reaction was subsequently observed in those sections.

2.3. Assessment of the thalamus

As previously published [10,29,30], the intralaminar nuclei of the thalamus can be distinguished from surrounding thalamic nuclei by their concentration of neuronal CalR (Fig. 1) and by their nuclear cytoarchitecture [21]. To assist with initial boundary identification for each region of interest, the general pattern of CalR immunoreactivity was visualized on a light box macroscopically using a series of slides, and the series of CalR-immunoreactive sections containing the nuclei of interest photographed using a digital camera at 1× magnification and printed using a colour printer. Prior to boundary delineation on an Olympus microscope at high magnification, each CalR-immunoreactive section was examined at 25× magnification to confirm the pattern of nuclei in that section. For the present study, the following intralaminar nuclei of the thalamus were detected. The parataenial thalamic nucleus (Pt) was characterized by sparsely distributed, faintly CalR-immunoreactive neurons. The anterior dorsal thalamic nucleus (AD) was clearly defined as an aggregation of small CalR-immunopositive neurons superior to Pt and adjacent to the anterior principal thalamic nucleus. The paraventricular thalamic nucleus (Pv) contained intensely stained CalR-immunoreactive neurons within a CalR-immunopositive neuropil located medial to the mediodorsal thalamic nucleus next to the ventricle. Occasionally irregularly shaped pockets of CalR-immunoreactive neurons appear on the lateral side of Pv and were included if they were contiguous. The cucullar thalamic nucleus (CU) was a discrete aggregate of intensely CalR-immunoreactive neurons and the central lateral thalamic nucleus (CL) was characterized by relatively sparsely distributed CalR-immunopositive neurons. The central medial thalamic nucleus (CeM) contained CalR-immunoreactive neurons within a variable background of CalR-immunoreactive neuropil. The centremedian (CM) and parafascicular (Pf) thalamic nuclei were considered together (CM/Pf). Boundary delineation was carried out at 100× magnification on a computer-motorized microscope using StereoInvestigator software (MicroBrightField, USA) to calculate nuclear size. The boundary outline of each thalamic nucleus of interest was drawn from each section in which it was present in each case and the volume of each intralaminar region of interest calculated by multiplying each nuclear cross-sectional area by the distance between sections. Repeated measures for a single operator gave <5% standard error of the mean for all nuclei areas, and repeated measures between two operators gave <10% standard error of the mean.

Within the nuclear boundaries identified, the severity of α-synuclein-immunopositive pathology was assessed semiquantitatively, as previously described [28,33]. Briefly, 0 = no Lewy bodies; 1 = sparse Lewy pathology; 2 = >1 Lewy body per low-power field and sparse Lewy neurites; 3 = ≥4 Lewy bodies per low-power field and scattered Lewy neurites; 4 = numerous Lewy bodies and Lewy neurites.

Within the nuclear boundaries identified, the number of neurons was determined using adjacent sections stained with cresyl violet with matching to cytoarchitectural regions and dissector protocols with the assistance of a computer-motorized microscope and StereoInvestigator software (MicroBrightField, USA), as previously described [15]. Due to the differing densities of neurons in each nucleus, dissector frames were spaced and sized specifically for each nucleus to give an accurate estimate of the total neuronal number. The frame size for AD, Pt, Pv, CU, CeM and CM/Pf was 140 μm × 140 μm spaced every 250 μm for AD, every 500 μm for Pt, every 750 μm for Pv, CU and CeM, and every 2000 μm for CM/Pf. For CL the frame size was 200 μm × 200 μm spaced every 1500 μm. Only neurons with a visible nucleolus in the section thickness that fell completely within the inclusion borders of the dissector frame were counted. Repeated measures of average cell number by a single rater gave <1% difference for all nuclei. The average number of cells counted in each dissector frame was divided by the volume of the frame (area × section thickness) to give an average neuronal density for each nucleus. This density was then multiplied by the volume of the whole nucleus to give total neuronal number.

2.4. Statistical analyses

Statistical analysis was performed with the Statview 5.0 program (Abacus Concepts, Berkeley, CA, USA) and *p* values less than 0.05 accepted as statistically significant. Analyses of variance and posthoc protected *t*-tests were used to determine any changes in the volume and neuron number in each intralaminar nucleus between diagnostic groups. Associations with pathological stage were assessed in cases with Lewy body diseases only using Spearman rank correlations. Relationships between pathological measures and age or disease durations were analysed using

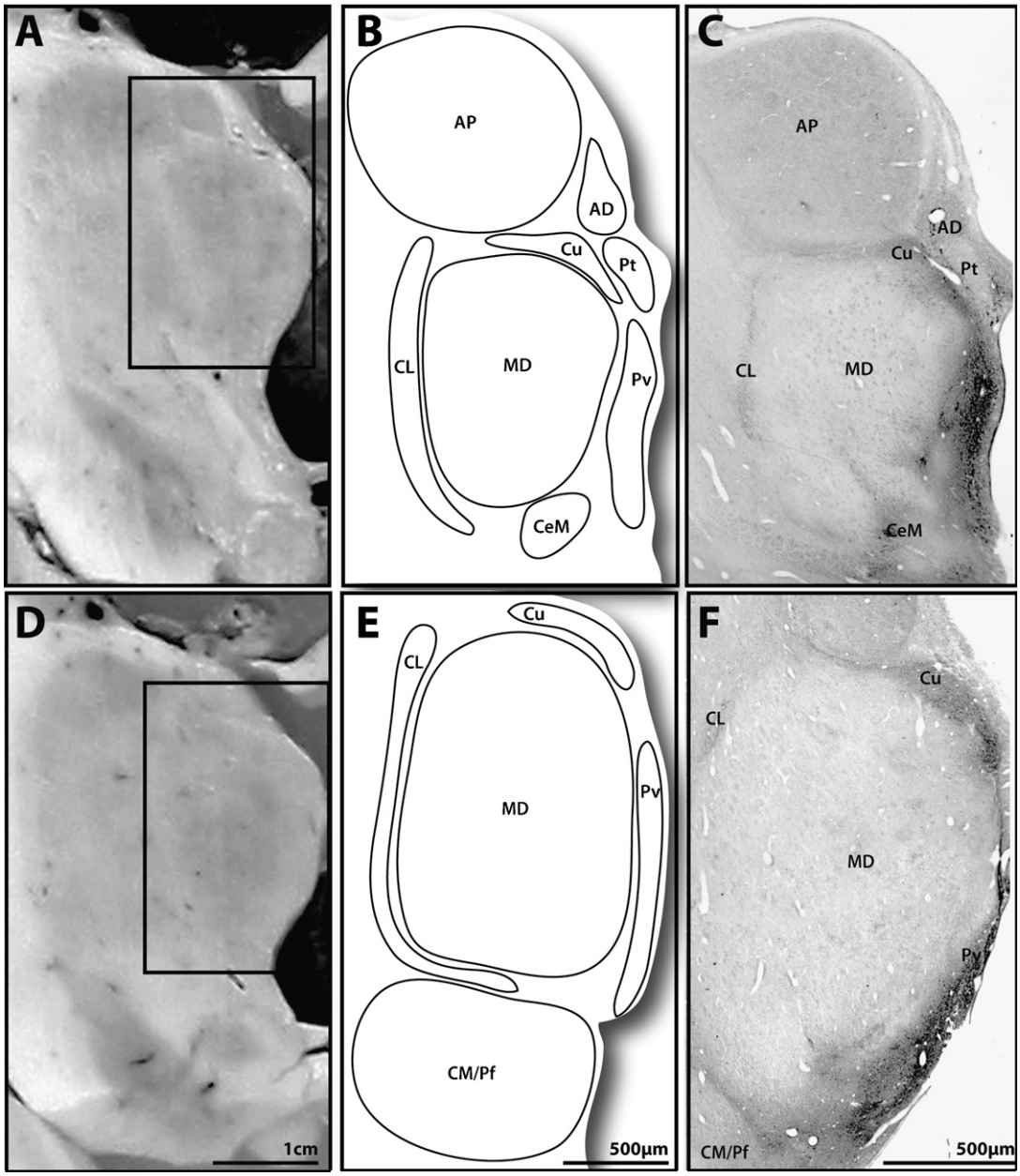

Fig. 1. Location and architecture of the intralaminar nuclei of the human thalamus at an anterior (A–C) and posterior (D–F) level. A and D show the entire thalamus in representative three mm sections with boxes indicating the architecture identified (B and E, respectively) from the 50 μm sections processed for calretinin immunohistochemistry (C and F, respectively). AD = anterior dorsal nucleus, AP = anterior principal nucleus, CeM = central medial nucleus, CL = central lateral, CM/Pf = centre-median and parafascicular nuclei, Cu = cucullar nucleus, MD = mediodorsal nucleus, Pt = parataenial nucleus, Pv = paraventricular nucleus, scale in D is the same for A, scale in E is the same for B, scale in F is the same for C.

linear regressions. Associations with the presence or absence of clinical variables were assessed in cases with Lewy body diseases only using Mann–Whitney *U* tests.

3. Results

3.1. Case variability

All Lewy body cases examined had Parkinsonism (Table 1). Fluctuations in consciousness occurred in 60% of the dementia cases studied (Table 1). Visual hallucinations were found in all PDD cases, and in 50% of cases with DLB (Table 1). DLB cases had a significantly shorter disease duration compared to both PD and PDD groups ($F_{3,21} = 6.0$, $p = 0.01$, Table 1). There was a progressive increase in the average PD stage between groups (Table 1) with most PD only cases having stage 4 Lewy body disease (subcortical concentration), while many PDD cases had stage 5 Lewy body disease (additional limbic infiltration), and all but one DLB case had stage 6 Lewy body disease (additional neocortical infiltration). Most DLB cases had cortical plaques (Table 1). Cases with Alzheimer's disease were excluded, and so only age-related neurofibrillary tangles were found in any of the cases studied (Table 1).

3.2. α-Synuclein pathology in the intralaminar thalamus in Lewy body diseases

As expected from previous studies [6,33], there was abnormal α-synuclein deposition in Lewy bodies, Lewy neurites, small intracellular deposits and other small intracellular and extracellular dot-

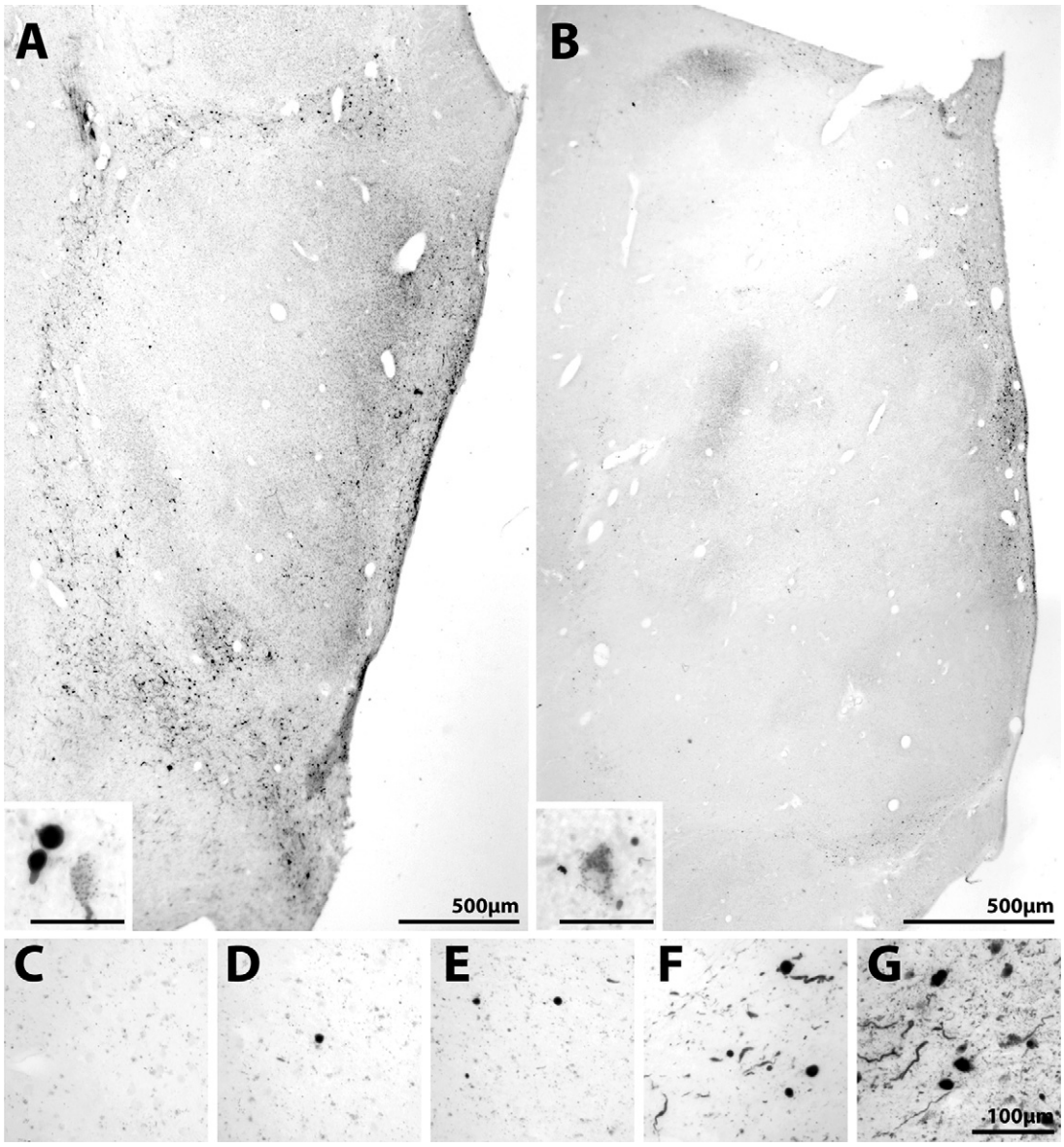

Fig. 2. Photomicrographs of a PDD case showing α-synuclein immunoreactivity in the medial nuclei of the human thalamus at an anterior (A) and posterior (B) level, as well as demonstrating the severity scale used to grade α-synuclein-positive pathology (C–G, see methods for details). α-Synuclein deposition can be seen in the paraventricular and central lateral intralaminar nuclei, with greater deposition occurring at anterior levels. Insets (scale = 50 µm) show α-synuclein-immunoreactive Lewy bodies (A) and neurons with dot-like intracytoplasmic α-synuclein deposits (A and B). (C) = Stage 0; (D) = Stage 1; (E) = Stage 2; (F) = Stage 3; (G) = Stage 4.

like deposits throughout the intralaminar thalamic nuclei in the patient groups examined (Fig. 2). No α-synuclein depositions were found in any of these regions in controls (data not shown). In contrast, the anterior and mediodorsal thalamic nuclei in the patients studied were distinguished by an absence of such α-synuclein immunoreactivity in cases with Lewy body disease (Fig. 2).

Within the intralaminar thalamic nuclei there was some variation in the severity of α-synuclein pathology. The degree of α-synuclein deposition was greatest in the anterior parts of the intralaminar nuclei and diminished progressively towards the posterior parts (Fig. 2). Comparison between Braak pathological stage of PD and the severity of α-synuclein deposition in the different intralaminar nuclei revealed significant correlations in two intralaminar nuclei, the central lateral ($\rho = 0.72$, $p = 0.007$) and paraventricular ($\rho = 0.64$, $p = 0.02$) nuclei. The severity of α-synuclein pathology in these nuclei is easily seen at low power (Fig. 2), with the pattern of α-synuclein pathology in the other intralaminar nuclei having greater variation across Braak Lewy body stages. α-Synuclein deposition correlated with age in the paraventricular nucleus only ($\rho = 0.58$, $p = 0.046$).

To determine whether the severity of α-synuclein deposition in different intralaminar nuclei associated with the presence of dementia, fluctuating consciousness and/or visual hallucinations, non-parametric Mann–Whitney *U* tests were performed. These analyses revealed an association between the presence of dementia and more severe α-synuclein deposition in only one intralaminar nucleus, the central lateral nucleus ($U = 4.5$, $p = 0.04$). There were no associations between the severity of α-synuclein deposition in the intralaminar thalamus and the presence of fluctuating consciousness or visual hallucinations.

3.3. Atrophy and cell loss in the intralaminar thalamus in Lewy body diseases

Cell loss was visibly apparent in a number of intralaminar thalamic nuclei in cases with Lewy body disease (Fig. 3). In general

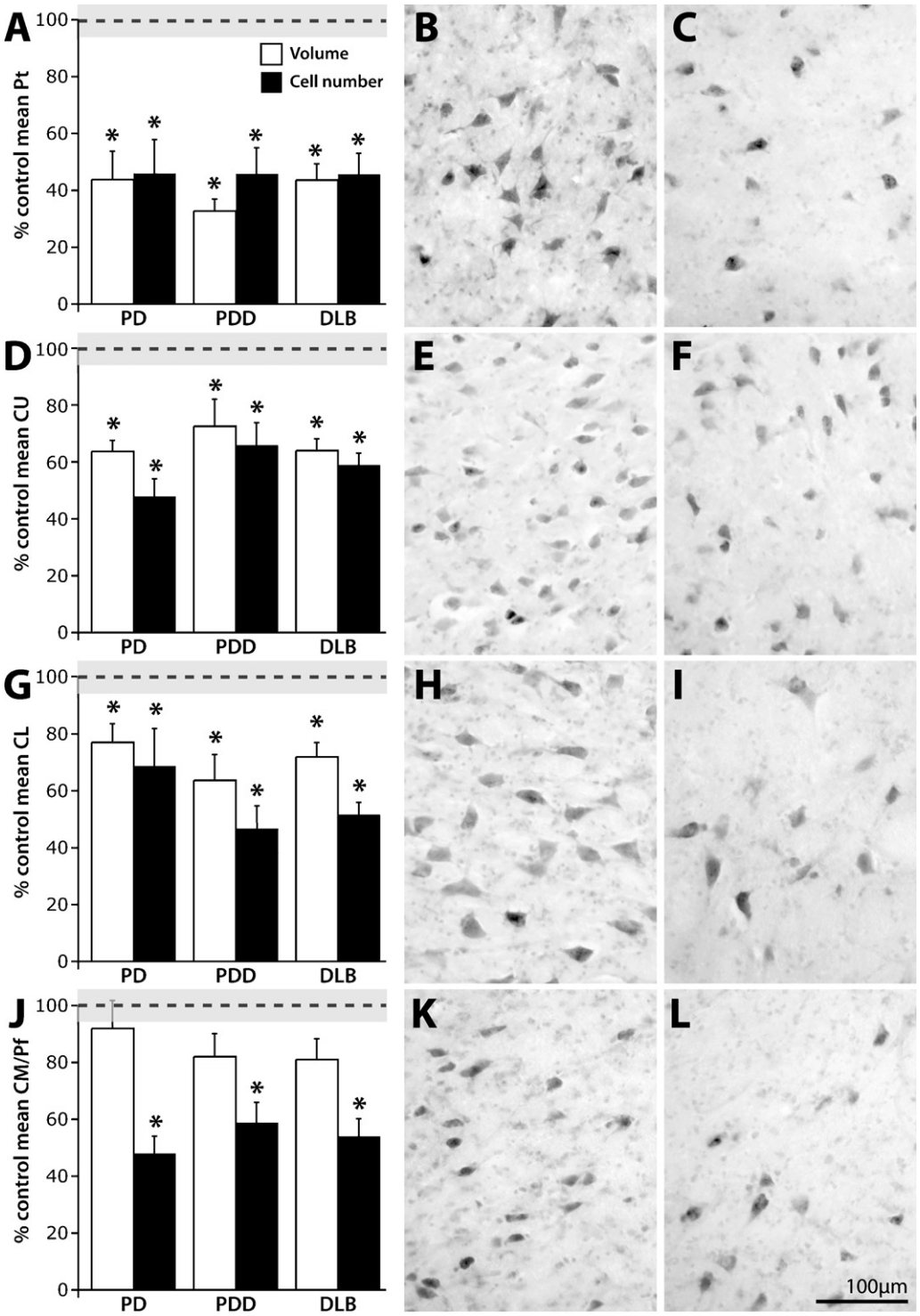

Fig. 3. Degeneration of four intralaminar thalamic nuclei was observed: parataenial (A–C), cucullar (D–F), central lateral (G–I) and the centre-median/parafascicular complex (J–L). Photomicrographs are taken from cresyl-violet stained 50 μm-thick sections in control (B, E, H, K) and DLB (C, F, I, L) cases. Neuronal loss is obvious in these regions. Graphs (A, D, G, J) show volume and cell number in disease groups as a percentage of the mean control value. Values different from controls ($p < 0.05$) using posthoc protected *t*-tests are identified with an asterisk. Bars represent standard error of the mean.

medially located nuclei were largely spared from neuronal degeneration (Table 2). As previously described in PD [19], there was neuronal loss (averaging 50–60%, $F_{3,21} = 7.9$, $p = 0.001$) without significant atrophy in the centre-median/parafascicular complex in PD as well as in the other Lewy body diseases (Table 2). This complex is the largest of the intralaminar regions located posteriorly and inferiorly (Fig. 1). Quantitation of the other smaller intralaminar nuclei revealed similar overall degeneration across all Lewy body disease groups in the laterally located central lateral nucleus (average 25–35% atrophy $F_{3,21} = 4.2$, $p = 0.017$; average 30–50% neuron loss $F_{3,21} = 6.8$, $p = 0.002$) and the superomedially located cucullar (average 25–35% atrophy $F_{3,21} = 6.9$, $p = 0.002$; average 30–50% neuron loss $F_{3,21} = 11.3$, $p = 0.000$) nuclei (Fig. 3 and Table 2). The greatest degeneration across groups was observed

Table 2
Comparison of quantitative data for the intralaminar nuclei of the thalamus in Lewy body diseases (unilateral mean ± standard deviations)

Nucleus	Variable	Control	PD	PDD	DLB	*p*
AD	α-Syn severity (0–4)	0	1.5 ± 2.1	2.0 ± 1.2	1.3 ± 0.5	0.5
	Volume (mm^3)	3.4 ± 0.3	3.4 ± 0.8	2.8 ± 1.0	3.5 ± 1.2	0.7
	Cell number (×1000)	20 ± 3	19 ± 5	15 ± 5	19 ± 6	0.4
Pt*	α-Syn severity (0–4)	0	1.0 ± 1.4	1.8 ± 0.5	2.1 ± 1.0	0.3
	Volume (mm^3)	9 ± 1	4 ± 2*	3 ± 1*	4 ± 1*	0.0001
	Cell number (×1000)	39 ± 5	18 ± 8*	18 ± 7*	18 ± 7*	0.0001
Pv	α-Syn severity (0–4)	0	2.0 ± 2.0	4.0 ± 0.0**	4.0 ± 0.0**	0.005
	Volume (mm^3)	18 ± 2	17 ± 5	16 ± 4	14 ± 2	0.17
	Cell number (×1000)	196 ± 52	163 ± 58	163 ± 37	142 ± 43	0.3
CU*	α-Syn severity (0–4)	0	1.3 ± 1.5	3.5 ± 0.6**	3.0 ± 0.7**	0.02
	Volume (mm^3)	11 ± 1	7 ± 1*	8 ± 2*	7 ± 2*	0.002
	Cell number (×1000)	90 ± 15	43 ± 11*	59 ± 15*	53 ± 12*	<0.0001
CL*	α-Syn severity (0–4)	0	1.7 ± 1.5	3.3 ± 0.5**	3.4 ± 0.7**	0.03
	Volume (mm^3)	47 ± 5	36 ± 8*	30 ± 10*	34 ± 7*	0.02
	Cell number (×1000)	206 ± 26	143 ± 67*	96 ± 38*	108 ± 26*	0.002
CeM	α-Syn severity (0–4)	0	1.5 ± 2.1	3.0 ± 0.8	3.7 ± 0.5**	0.02
	Volume (mm^3)	15 ± 2	14 ± 4	12 ± 3	10 ± 4	0.12
	Cell number (×1000)	115 ± 37	91 ± 24	77 ± 21	75 ± 29	0.13
CM/Pf*	α-Syn severity (0–4)	0	0.3 ± 0.6	1.5 ± 0.6	1.4 ± 0.9	0.1
	Volume (mm^3)	166 ± 25	152 ± 44	136 ± 30	134 ± 37	0.4
	Cell number (×1000)	871 ± 195	416 ± 112*	518 ± 142*	466 ± 161*	0.001

* Different from control using posthoc protected *t*-tests, $p < .05$.
** Different from PD using posthoc protected *t*-tests, $p < .05$.

in the parataenial nucleus which had approximately 55% cell loss ($F_{3,21} = 9.5$, $p = 0.000$) and was reduced in volume on average by 55–65% ($F_{3,21} = 15.7$, $p = 0.000$) compared with controls (Fig. 3 and Table 2). There was no significant atrophy or cell loss in the medially located paraventricular and central medial intralaminar nuclei, or in the superoanteriorly located anterior dorsal nucleus (Table 2).

To assess whether the severity of α-synuclein pathology in the intralaminar nuclei related to cell loss, correlations to the volume and neuronal number in these regions were assessed. These analyses revealed an association between increasingly severe α-synuclein deposition and atrophy in only one region, the centre-median/parafascicular intralaminar complex ($\rho = -0.57$, $p = 0.03$). Disease duration was associated with increasing neuronal loss in the parataenial nucleus ($\rho = -0.60$, $p = 0.04$).

To determine whether the degeneration in different intralaminar nuclei associated with the presence of dementia, fluctuating consciousness and/or visual hallucinations, non-parametric Mann–Whitney *U* tests were performed. These analyses revealed an average volume loss of 18% in the cucullar nucleus in those Lewy body disease cases with visual hallucinations compared to those without ($U = 26$, $p = 0.04$). There was no association between volume or cell loss in the intralaminar nuclei of the thalamus in Lewy body disease and the presence of fluctuating consciousness or dementia.

4. Discussion

This is the first time that damage to the neuronal populations of the intralaminar thalamic nuclei has been quantified across the different types of Lewy body diseases. The degree of α-synuclein immunoreactive inclusions previously demonstrated in PD [33] would suggest considerable degeneration of nuclei and neurons, which our data supports. Significant atrophy and neuronal loss was noted in all cases in the parataenial, cucullar, central lateral nuclei and significant neuronal loss in the centre-median/parafascicular complex. The degeneration of the centre-median/parafascicular complex is similar to that previously reported in PD [19], confirming this finding in a separate sample, and showing similar degeneration in cases with PDD and DLB. It is of interest that the loss of intralaminar neurons in Lewy body cases with dementia was not greater than in those without dementia, as previous predictions were for more substantive neurodegeneration associated with dementia [33]. However, given the key role these thalamic nuclei play in integration of higher order cognitive functions [38], this degeneration is likely to have other clinical impact.

There are only a limited number of previous studies that have assessed the consequences of degeneration in intralaminar nuclei, although the similar degree of atrophy in all our cases with Parkinsonism suggests some association to this core feature. Similar atrophy and neuronal loss of the central lateral and centre-median/parafascicular nuclei has been found in head injured patients who remain in a vegetative state [25]. The paramedian artery supplies these nuclei, and infarction of this vessel and degeneration of these nuclei leads to marked changes in arousal, in particular decreased and fluctuating levels of consciousness [36]. These types of injury have a large impact on these neuronal populations with marked clinical consequences. Significant degeneration of particular thalamic intralaminar nuclei in Lewy body patients with fluctuations in consciousness was not detected in this study. However, in Lewy body patients there is selective degeneration of only particular intralaminar neurons immunopositive for different calcium-binding proteins [19]. Various types of intralaminar neurons have different projections and functional responses [22], with the neurons affected in the centre-median/parafascicular complex more likely to have basal ganglia projections [19]. The more discrete degeneration of particular types of intralaminar neurons in Lewy body disease may account for the lack of correlation to the severe fluctuations in consciousness assessed in the present study.

Greater atrophy of the intralaminar cucullar nucleus was found to be associated with the presence of visual hallucinations in cases with Lewy body disease. This thalamic region has pathological alteration in Alzheimer's disease [5,32] and spinocerebellar ataxia

[34,35], but it is difficult to pick the clinical feature in common in these disorders. Despite a number of detailed descriptions of this thalamic nucleus [3,7,18], its connections and function remain elusive, and it has generally been considered similar to central intralaminar nuclei [5,21]. Although speculative, this region may be connected with the amygdala, another region with pathology associated with visual hallucinations in Lewy body diseases [17]. The amygdala has significant projections to the mediodorsal nucleus (reviewed in [12]), and given the close association of the cucullar to this nucleus as part of the dorsomedial envelope [18,37], amygdala denervation of the cucullar intralaminar nucleus may be the pathological substrate for the clinical association noted.

More substantial α-synuclein deposition in those intralaminar nuclei with neuronal loss was associated with the presence of dementia in Lewy body diseases. A previous pathological study in PD and PDD showed a similar increase in α-synuclein deposition in the intralaminar thalamus in PDD, and predicted that this increased pathology would occur in DLB also [6,33]. Of all the intralaminar nuclei assessed, the severity of α-synuclein deposition in the central lateral nucleus most closely associated with the presence of dementia. The central lateral nucleus has strong connections with cingulate cortex, particularly the anterior cingulate region, and is considered important for cognitive awareness and working memory [38].

Several pathological changes identified in the intralaminar nuclei correlated with indices of disease progression. The Braak pathological stage of PD correlated with the severity of α-synuclein pathology in the central lateral and paraventricular nuclei. Increasing α-synuclein pathology correlated with increasing atrophy in the centre-median/parafascicular complex. Changes in the basal ganglia innervation of these regions over time may account for these relationships. Disease duration correlated with increasing neuronal loss in the most severely affected intralaminar region, the parataenial nucleus. The parataenial nucleus mainly targets infralimbic and prelimbic cortices [38], with some projections to anterior cingulate cortices [39], potentially impacting on executive function.

We have demonstrated that several intralaminar nuclei have significant neuronal loss in Lewy body diseases, with considerable α-synuclein deposition associated with this neurodegeneration. The parataenial, cucullar and central lateral nuclei undergo atrophy and neuronal loss, with significant cell loss also found in the centre-median/parafascicular complex. The centrality of these thalamic nuclei in networks underlying movement and cognitive functions suggests a key role in the pathological changes leading to the clinical deficits in these disorders.

References

[1] D. Aarsland, R. Perry, A. Brown, J.P. Larsen, C. Ballard, Neuropathology of dementia in Parkinson's disease: a prospective, community-based study, Ann. Neurol. 58 (2005) 773–776.

[2] N. Adachi, T. Watanabe, H. Matsuda, T. Onuma, Hyperperfusion in the lateral temporal cortex, the striatum and the thalamus during complex visual hallucinations: single photon emission computed tomography findings in patients with Charles Bonnet syndrome, Psychiatry Clin. Neurosci. 54 (2000) 157–162.

[3] J. Andrew, E.S. Watkins, A Stereotaxic Atlas of the Human Thalamus and Adjacent Structures; A Variability Study, Williams and Wilkins, Baltimore, 1969.

[4] M. Baba, S. Nakajo, P.H. Tu, T. Tomita, K. Nakaya, V.M. Lee, J.Q. Trojanowski, T. Iwatsubo, Aggregation of alpha-synuclein in Lewy bodies of sporadic Parkinson's disease and dementia with Lewy bodies, Am. J. Pathol. 152 (1998) 879–884.

[5] H. Braak, E. Braak, Alzheimer's disease affects limbic nuclei of the thalamus, Acta Neuropathol. (Berl) 81 (1991) 261–268.

[6] H. Braak, K. Del Tredici, H. Bratzke, J. Hamm-Clement, D. Sandmann-Keil, U. Rub, Staging of the intracerebral inclusion body pathology associated with idiopathic Parkinson's disease (preclinical and clinical stages), J. Neurol. 249 (Suppl. (3)) (2002), III/1–5.

[7] A. Dewulf, Anatomy of the Normal Human Thalamus: Topometry and Standardized Nomenclature, Elsevier Publishing Company, Amsterdam, 1971.

[8] D.W. Dickson, Dementia with Lewy bodies: neuropathology, J. Geriatr Psychiatry Neurol. 15 (2002) 210–216.

[9] J.E. Duda, B.I. Giasson, M.E. Mabon, V.M. Lee, J.Q. Trojanowski, Novel antibodies to synuclein show abundant striatal pathology in Lewy body diseases, Ann. Neurol. 52 (2002) 205–210.

[10] M. Fortin, M.C. Asselin, P.V. Gould, A. Parent, Calretinin-immunoreactive neurons in the human thalamus, Neuroscience 84 (1998) 537–548.

[11] D.J. Gelb, E. Oliver, S. Gilman, Diagnostic criteria for Parkinson disease, Archives of Neurology 56 (1999) 33–39.

[12] P. Gloor (Ed.), The Temporal Lobe and Limbic System, Oxford University Press, New York, 1997, pp. 674–677.

[13] R. Goldmann Gross, A. Siderowf, H.I. Hurtig, Cognitive impairment in Parkinson's disease and dementia with Lewy bodies: a spectrum of disease, Neurosignals 16 (2008) 24–34.

[14] G.M. Halliday, V. Macdonald, J.M. Henderson, A comparison of degeneration in motor thalamus and cortex between progressive supranuclear palsy and Parkinson's disease, Brain 128 (2005) 2272–2280.

[15] A.J. Harding, G.M. Halliday, K. Cullen, Practical considerations for the use of the optical dissector in estimating neuronal number, J. Neurosci. Methods 51 (1994) 83–89.

[16] A.J. Harding, G.A. Broe, G.M. Halliday, Visual hallucinations in Lewy body disease relate to Lewy bodies in the temporal lobe, Brain 125 (2002) 391–403.

[17] A.J. Harding, E. Stimson, J.M. Henderson, G.M. Halliday, Clinical correlates of selective pathology in the amygdala of patients with Parkinson's disease, Brain 125 (2002) 2431–2445.

[18] R. Hassler, Anatomy of the thalamus, in: G. Schaltenbrand, P. Bailey (Eds.), Introduction to Stereotaxis with an Atlas of the Human Brain, Thieme, Stuttgart, 1959, pp. 230–290.

[19] J.M. Henderson, K. Carpenter, H. Cartwright, G.M. Halliday, Degeneration of the centré median-parafascicular complex in Parkinson's disease, Ann. Neurol. 47 (2000) 345–352.

[20] J.M. Henderson, K. Carpenter, H. Cartwright, G.M. Halliday, Loss of thalamic intralaminar nuclei in progressive supranuclear palsy and Parkinson's disease: clinical and therapeutic implications, Brain 123 (2000) 1410–1421.

[21] T. Hirai, E.G. Jones, A new parcellation of the human thalamus on the basis of histochemical staining., Brain Res.—Brain Res. Rev. 14 (1989) 1–34.

[22] E.G. Jones, Chemically defined parallel pathways in the monkey auditory system, Ann. N. Y. Acad. Sci. 999 (2003) 218–233.

[23] S. Kinomura, J. Larsson, B. Gulyas, P.E. Roland, Activation by attention of the human reticular formation and thalamic intralaminar nuclei, Science 271 (1996) 512–515.

[24] C.F. Lippa, J.E. Duda, M. Grossman, H.I. Hurtig, D. Aarsland, B.F. Boeve, D.J. Brooks, D.W. Dickson, B. Dubois, M. Emre, S. Fahn, J.M. Farmer, D. Galasko, J.E. Galvin, C.G. Goetz, J.H. Growdon, K.A. Gwinn-Hardy, J. Hardy, P. Heutink, T. Iwatsubo, K. Kosaka, V.M. Lee, J.B. Leverenz, E. Masliah, I.G. McKeith, R.L. Nussbaum, C.W. Olanow, B.M. Ravina, A.B. Singleton, C.M. Tanner, J.Q. Trojanowski, Z.K. Wszolek, DLB and PDD boundary issues: diagnosis, treatment, molecular pathology, and biomarkers, Neurology 68 (2007) 812–819.

[25] W.L. Maxwell, M.A. MacKinnon, D.H. Smith, T.K. McIntosh, D.I. Graham, Thalamic nuclei after human blunt head injury, J. Neuropathol. Exp. Neurol. 65 (2006) 478–488.

[26] I. McKeith, Dementia with Lewy bodies and Parkinson's disease with dementia: where two worlds collide, Pract. Neurol. 7 (2007) 374–382.

[27] I.G. McKeith, U.P. Mosimann, Dementia with Lewy bodies and Parkinson's disease, Parkinsonism Relat. Disord. 10 (Suppl. (1)) (2004) S15–S18.

[28] I.G. McKeith, D.W. Dickson, J. Lowe, M. Emre, J.T. O'Brien, H. Feldman, J. Cummings, J.E. Duda, C. Lippa, E.K. Perry, D. Aarsland, H. Arai, C.G. Ballard, B. Boeve, D.J. Burn, D. Costa, T. Del Ser, B. Dubois, D. Galasko, S. Gauthier, C.G. Goetz, E. Gomez-Tortosa, G. Halliday, L.A. Hansen, J. Hardy, T. Iwatsubo, R.N. Kalaria, D. Kaufer, R.A. Kenny, A. Korczyn, K. Kosaka, V.M. Lee, A. Lees, I. Litvan, E. Londos, O.L. Lopez, S. Minoshima, Y. Mizuno, J.A. Molina, E.B. Mukaetova-Ladinska, F. Pasquier, R.H. Perry, J.B. Schulz, J.Q. Trojanowski, M. Yamada, Diagnosis and management of dementia with Lewy bodies: third report of the DLB Consortium, Neurology 65 (2005) 1863–1872.

[29] A. Morel, M. Magnin, D. Jeanmonod, Multiarchitectonic, stereotactic atlas of the human thalamus, J. Comp. Neurol. 387 (1997) 588–630.

[30] M.C. Münkle, H.J. Waldvogel, R.L. Faull, The distribution of calbindin, calretinin and parvalbumin immunoreactivity in the human thalamus, J. Chem. Neuroanat. 19 (2000) 155–173.

[31] B. Ramirez-Ruiz, C. Junque, M.J. Marti, F. Valldeoriola, E. Tolosa, Cognitive changes in Parkinson's disease patients with visual hallucinations, Dement Geriatr. Cogn. Disord. 23 (2007) 281–288.

[32] U. Rüb, K. Del Tredici, D. Del Turco, H. Braak, The intralaminar nuclei assigned to the medial pain system and other components of this system are early and progressively affected by the Alzheimer's disease-related cytoskeletal pathology, J. Chem. Neuroanat. 23 (2002) 279–290.

[33] U. Rüb, K. Del Tredici, C. Schultz, E. Ghebremedhin, R.A. de Vos, E. Jansen Steur, H. Braak, Parkinson's disease: the thalamic components of the limbic loop are severely impaired by alpha-synuclein immunopositive inclusion body pathology, Neurobiol. Aging 23 (2002) 245–254.

[34] U. Rüb, D. Del Turco, K. Del Tredici, R.A. de Vos, E.R. Brunt, G. Reifenberger, C. Seifried, C. Schultz, G. Auburger, H. Braak, Thalamic involvement in a spinocerebellar ataxia type 2 (SCA2) and a spinocerebellar ataxia type 3 (SCA3) patient, and its clinical relevance, Brain 126 (2003) 2257–2272.

[35] U. Rüb, D. Del Turco, K. Burk, G.O. Diaz, G. Auburger, M. Mittelbronn, K. Gierga, E. Ghebremedhin, C. Schultz, L. Schols, J. Bohl, H. Braak, T. Deller, Extended pathoanatomical studies point to a consistent affection of the thalamus in spinocerebellar ataxia type 2, Neuropathol. Appl. Neurobiol. 31 (2005) 127–140.
[36] J.D. Schmahmann, Vascular syndromes of the thalamus, Stroke 34 (2003) 2264–2278.
[37] H. Strenge, [The dorsomedial envelope in the human thalamus. A pigment architectonic study], Z. Mikrosk. Anat. Forsch. 90 (1976) 893–907.
[38] Y.D. Van der Werf, M.P. Witter, H.J. Groenewegen, The intralaminar and midline nuclei of the thalamus. Anatomical and functional evidence for participation in processes of arousal and awareness, Brain Res. Brain Res. Rev. 39 (2002) 107–140.
[39] B.A. Vogt, D.N. Pandya, D.L. Rosene, Cingulate cortex of the rhesus monkey: I. Cytoarchitecture and thalamic afferents, J. Comp. Neurol. 262 (1987) 256–270.