

**DIFFERENT MOLECULAR PATHOLOGIES RESULT IN SIMILAR
SPATIAL PATTERNS OF CELLULAR INCLUSIONS IN
NEURODEGENERATIVE DISEASE: A COMPARATIVE STUDY
OF EIGHT DISORDERS**

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Running Head: Spatial patterns of inclusions

Abstract

Recent research suggests cell to cell transfer of pathogenic proteins such as tau and α -synuclein may play a role in neurodegeneration. Pathogenic spread along neural pathways may give rise to specific spatial patterns of the neuronal cytoplasmic inclusions (NCI) characteristic of these disorders. Hence, the spatial patterns of NCI were compared in four tauopathies, viz., Alzheimer's disease (AD), Pick's disease (PiD), corticobasal degeneration (CBD), and progressive supranuclear palsy (PSP), two synucleinopathies, viz., dementia with Lewy bodies (DLB) and multiple system atrophy (MSA), the 'fused in sarcoma' (FUS)-immunoreactive inclusions in neuronal intermediate filament inclusion disease (NIFID), and the transactive response (TAR) DNA-binding protein (TDP-43)-immunoreactive inclusions in frontotemporal lobar degeneration, a TDP-43 proteinopathy (FTLD-TDP). Regardless of molecular group or morphology, NCI were most frequently aggregated into clusters, the clusters being regularly distributed parallel to the pia mater. In a significant proportion of regions, the regularly distributed clusters were in the size range 400 - 800 μ m, approximating to the dimension of cell columns associated with the cortico-cortical pathways. The data suggest that cortical NCI in different disorders exhibit a similar spatial pattern in the cortex consistent with pathogenic spread along anatomical pathways. Hence, treatments designed to protect the cortex from neurodegeneration may be applicable across several different disorders.

Key Words: Tauopathy; Synucleinopathy; FUS proteinopathy; TDP-43 proteinopathy; Spatial patterns; Neuronal cytoplasmic inclusions (NCI); Cell to cell transfer

Introduction

The formation of neuronal cytoplasmic inclusions (NCI), which vary in morphology and molecular composition, is a characteristic feature of many neurodegenerative disorders (Armstrong et al 2008). These include neurofibrillary tangles (NFT) in Alzheimer's disease (AD) (Armstrong 1993a) and progressive supranuclear palsy (PSP) (Armstrong et al 2007), Pick bodies (PB) in Pick's disease (PiD) (Armstrong et al 1998), tau-immunoreactive neuronal inclusions in corticobasal degeneration (CBD) (Armstrong et al 2009), Lewy bodies (LB) in dementia with Lewy bodies (DLB) (Armstrong et al 1997), and NCI in multiple system atrophy (MSA) (Armstrong et al 2006a), neuronal intermediate filament inclusion disease (NIFID) (Cairns et al 2004a, 2004b, 2004c, Armstrong et al 2006b), and frontotemporal lobar degeneration (FTLD) (Armstrong et al 2010).

The majority of inclusions are characterized by either abnormal aggregates of the microtubule-associated protein (MAP) tau (tauopathies), e.g., AD, PiD, argyrophilic grain disease (AGD) (Saito et al 2004), PSP, CBD, and frontotemporal dementia and parkinsonism linked to chromosome 17 (FTDP-17) (Reed et al 2001), or α -synuclein (synucleinopathies), e.g., Parkinson's disease (PD), DLB, and MSA (Goedert et al 2001). Within the tauopathies, the molecular composition of tau varies, PiD being characterised by tau with three microtubule repeats (3R tau) while PSP and CBD are composed of four-repeat (4R) tau (Dickson 1999, Morris et al 2002). In AD, NFT are characterised by both 3R and 4R tau and composition also varies markedly depending on whether NFT are intracellular (I-NFT) or extracellular (E-NFT) (Yamaguchi et al 1991). In addition to tau and α -synuclein, other molecular pathologies have been recently identified. Hence, NCI in FTLD are characterised by transactive response (TAR) DNA-binding protein (TDP-43)-immunoreactive inclusions and therefore, FTLD is a TDP-43 proteinopathy (FTLD-TDP) (Neumann et al 2006, Armstrong et al 2010) while NCI in NIFID are characterised by 'fused in sarcoma' (FUS)-immunoreactivity (Yokata et al 2008, Neumann et al 2009, Armstrong et al 2011a, 2011b). NCI also exhibit different morphologies but the majority are either flame-shaped, e.g., NFT in AD, CBD, and PSP or globose, e.g., PiD, DLB, and NIFID. In contrast, a variety of inclusion morphologies are often present in FTLD-TDP

including rounded, spicular, or skein-like inclusions (Davidson et al 2007, Yaguchi et al 2004).

The relationship between the various types of inclusion and the disease process itself is controversial. At least three hypotheses could be proposed to explain the relationship between the appearance of a particular inclusion and disease pathogenesis. First, that the pathological inclusions are the primary cause of the observed neurodegeneration ('causal hypothesis'), e.g., deposition of abnormal tau or α -synuclein may be the key event causing neuronal degeneration. Second, that the lesions are a consequence of neurodegenerative processes but not directly related to their cause ('reaction hypothesis'), e.g., in PiD and CBD, a pathological process unrelated to tau deposition could cause the degeneration of neurons and result in the aggregation of tau. Third, inclusions could be a consequence of the neurodegeneration but their formation may be directly linked to the primary pathological mechanism ('causal/reaction/hypothesis'). Hence, the presence of α -synuclein within cells could initiate the process of degeneration of neuronal pathways and then the subsequent formation of abnormal aggregates in the form of inclusions.

Hardy and Gwinn-Hardy (1998) suggested that there may be relatively few cellular pathways leading to cell death in different disorders, and as a consequence, diseases classified within different molecular groups may have pathological mechanisms in common. Recent research suggests that pathogenic proteins such as tau and α -synuclein can be secreted from cells, enter other cells, and seed small intracellular aggregates within these cells (Goedert et al 2010, Steiner et al 2011). This raises the possibility, first suggested by Braak and colleagues with reference to PD (Hawkes et al 2007), that pathogenic agents may transfer along unmyelinated axons to basal areas of brain, the brain stem and then to the cerebral cortex. If pathogenic proteins can spread from cell to cell in the cortex, then the resulting NCI may exhibit a spatial pattern which reflects this spread. Previous studies have suggested non-random distributions of NCI in the cerebral cortex of various disorders, the inclusions often exhibiting a distinct clustering pattern (Armstrong et al 2001). The present study compared the spatial patterns of NCI in eight neurodegenerative disorders including four tauopathies (AD, PiD, CBD, PSP), two synucleinopathies (DLB, MSA), FTLN-

TDP, and NIFID to determine whether the spatial patterns of the inclusions were consistent with cell to cell spread along anatomical pathways.

Materials and Methods

Cases

With the exception of NIFID and FTLD-TDP, all cases were obtained from the Brain Bank, Department of Neuropathology, Institute of Psychiatry, King's College, London, UK. NIFID cases (N = 10) (Armstrong et al 2006b) were obtained from centres in Canada, Norway, Spain, Japan (one case from each), and from France, the UK, and the USA (two cases from each) and have been described in detail by Cairns et al (2004c). FTLD-TDP cases (N = 15) were obtained from Harvard Brain Tissue Resource Centre, Belmont, MA (2 cases), Vancouver General Hospital (6 cases), University of Pittsburgh (3 cases), Washington University, St Louis (3 cases), and the University of California, Davis (1 case) (Armstrong et al 2010). Demographic data and diagnostic criteria for the various disorders are listed in Table 1. Stage of disease was represented by neurofibrillary tangle score (range: 0, 1 - 6) (Braak et al 2006) with the exception of DLB in which a PD staging scale for LB was used (range: 0, 1-6) (Braak et al 2004).

Tissue preparation

After death, the consent of the next of kin was obtained for brain removal, following local Ethical Committee procedures and the 1999 Declaration of Helsinki (as modified Edinburgh, 2000). Brain tissue was preserved in buffered 10% formalin or 4% paraformaldehyde. Tissue blocks were taken from various cortical areas (Table 1) including the superior frontal gyrus (SFG) (B8), the superior parietal lobule (SPL) (B7a/7b), the inferior temporal gyrus (ITG) (B22), the parahippocampal gyrus (PHG) (B28), and the occipital cortex (B17). Brain material was fixed in 10% phosphate buffered formal-saline, and embedded in paraffin wax. Immunohistochemistry (IHC) was performed on 6 - 8µm sections using appropriate antibodies (Table 1). Sections were counterstained with haematoxylin.

Morphometric methods

In each region of the cerebral cortex examined, NCI were counted along a strip of tissue (3200 to 6400 μm in length) located parallel to the pia mater, using 250 x 50 μm sample fields arranged contiguously (Armstrong 2003) (Fig 1). The sample fields were located both in the upper (approximating to laminae II/III) and lower (approximating to laminae V/VI) cortex, the short edge of the sample field being orientated parallel with the pia mater and aligned with guidelines marked on the slide. The number of NCI present in each sample field was counted.

Data analysis

The data were analysed by spatial pattern analysis (Armstrong 1993b, 1997, 2006, 2007). This method uses the variance-mean ratio (V/M) to determine whether NCI were distributed randomly (V/M = 1), regularly (V/M < 1), or were clustered (V/M > 1) along a strip of tissue. Counts of NCI in adjacent sample fields were added together successively to provide data for increasing field sizes, e.g., 50 x 250 μm , 100 x 250 μm , 200 x 250 μm etc., up to a size limited by the length of the strip sampled. V/M was then plotted against field size to determine whether the clusters of NCI were regularly or randomly distributed and to estimate the mean cluster size parallel to the tissue boundary. A V/M peak indicates the presence of regularly spaced clusters while an increase in V/M to an asymptotic level suggests the presence of randomly distributed clusters. The statistical significance of a peak was tested using 't' tests (Armstrong 1997). Spatial patterns of inclusions in the various regions were classified initially into four categories: random, uniform or regular, regularly distributed clusters, and large clusters ($\geq 1600\mu\text{m}$). The frequency of regions in which regularly distributed clusters were in the size range 400 – 800 μm was also determined. In addition, a more complex spatial pattern was evident in some areas in which smaller clusters of inclusions were aggregated into large superclusters and frequency of this pattern was determined for each disorder. The effect of inclusion morphology, type of disorder, and molecular pathology on the frequency of the different types of spatial pattern was tested using chi-square (χ^2) contingency table analysis.

In addition, the various disorders were compared using principal components analysis (PCA) (Armstrong et al 2010) (STATISTICA software, Statsoft Inc., 2300 East 14th St, Tulsa, Ok, 74104, USA). The result of a PCA is a scatter plot of the eight disorders in relation to the extracted PC in which the distance between disorders reflects their similarity or dissimilarity based on the frequency of the different types of spatial patterns of their respective inclusions.

Finally, mean cluster size of NCI was compared between disorders using a one-way analysis of variance (ANOVA) (STATISTICA software, Statsoft Inc., 2300 East 14th St, Tulsa, Ok, 74104, USA) followed by Scheffé's *post-hoc* test. In each disorder, the correlation (Pearson's 'r') between cluster size of the inclusions, disease duration, and disease stage were tested.

Results

Examples of the spatial patterns exhibited by various NCI in different disorders are shown in Figs 2 - 4. Fig 2 shows a random distribution of tau-immunoreactive neuronal inclusions in CBD, while Fig 3 shows a small cluster of LB, approximately 70 μ m in diameter in DLB, and Fig 4 a larger cluster of NCI in NIFID.

A comparison of the spatial patterns exhibited by the 3R tau-immunoreactive PB and α -synuclein-immunoreactive LB in the ITG of cases of PiD and DLB respectively is shown in Fig 5. Both types of inclusion are distributed in regular clusters. Hence, LB in DLB exhibited two V/M peaks, the first at a field size of 400 μ m and the second at 1600 μ m, suggesting the presence of clusters of LB, 400 μ m in diameter, regularly distributed parallel to the pia mater and aggregated into larger clusters 1600 μ m in diameter. By contrast, PB in PiD exhibited a single V/M peak at a field size of 1600 μ m suggesting regularly distributed clusters of PB, 1600 μ m in diameter.

A summary of the frequencies of the four types of spatial patterns exhibited by NCI in the upper and lower cortex of the eight disorders is shown in Table 2. Most frequently, NCI were clustered, mean cluster size being in the range 50 - 1600 μ m, and the clusters were regularly distributed parallel to the pia mater. This spatial pattern varied in frequency in different disorders from 33% of gyri studied in MSA to

74% in NIFID. Larger-scale clustering, in which the NCI occurred in clusters of at least 1600 μ m in diameter, but without regular spacing, was also present especially in PiD, CBD, and DLB. In some disorders, viz., PSP, MSA, and FTLN-TDP, the NCI were randomly distributed in a significant number of gyri.

Chi-square (χ^2) contingency tests (Table 2) suggested: (1) the frequency of the different spatial patterns of NCI was similar in the tauopathies and synucleinopathies ($\chi^2 = 0.16$, 3DF, $P > 0.05$), (2) within the tauopathies, the spatial pattern of the PB in PiD was similar to that of the NCI in CBD ($\chi^2 = 2.52$, 3DF, $P > 0.05$) but NFT in PSP exhibited a higher proportion of random distributions than in AD ($\chi^2 = 9.47$, 2DF, $P < 0.01$), (3) within the tauopathies, disorders characterised by 3R tau (PiD) had similar spatial patterns of NCI to those characterised by 4R tau (PSP, CBD) ($\chi^2 = 5.94$, $P > 0.05$, 3DF, $P > 0.05$), (4) within the synucleinopathies, there was a greater frequency of random distributions of NCI in MSA compared with LB in DLB ($\chi^2 = 27.36$, 3DF, $P < 0.001$), (5) there were significant differences in spatial patterns of NCI between the tauopathies and synucleinopathies compared with NIFID ($\chi^2 = 13.96$, 3DF, $P < 0.001$) and FTLN-TDP ($\chi^2 = 28.87$, 3DF, $P < 0.001$), smaller-scale clustering being more frequent in the latter two disorders, and (6) spatial patterns of NCI in disorders with predominantly cortical flame-shaped inclusions (AD, PSP, CBD) were similar to those in disorders with predominantly round or oval inclusions (PiD, DLB, NIFID) ($\chi^2 = 7.34$, 3DF, $P > 0.05$). There were no significant differences in frequency of spatial patterns between upper and lower regions of the cortex.

A summary of the number of regions in each disorder in which first, regularly distributed clusters were in the size range 400 – 800 μ m and second, a more complex spatial pattern was present is shown in Table 3. In a significant proportion of gyri in all disorders, with the exception of MSA, the regularly distributed clusters of inclusions were in the size range 400 - 800 μ m. A more complex spatial pattern in which smaller clusters were aggregated into larger superclusters is present in a small number of regions in all disorders with the exception of MSA and PSP.

Mean cluster size of NCI (Fig 6), averaged over all cortical areas, varied between the different disorders ($F = 14.73$, $P < 0.001$), *post-hoc* tests suggesting that: (1) cluster sizes were essentially similar in AD, PiD, CBD, and DLB and (2) clusters were significantly larger in AD, PiD, CBD, and DLB than in PSP, MSA, NIFID, and FTLD-TDP. No statistically significant correlations were observed between cluster size of inclusions and either disease duration or stage.

A PCA of the data, based on the frequencies of the different types of spatial patterns, is shown in Fig 7. The first two PC accounted for 96% of the total variance in the data (PC1 = 69%, PC2 = 27%). A plot of the various disorders in relation to the first two PC (PC1 and PC2) suggested that spatial patterns of NCI in AD, CBD, PiD, DLB, and NIFID were similar, viz., in a significant proportion of regions, the inclusions were clustered and regularly distributed. However, in PSP and MSA the inclusions were also randomly distributed in a significant number of areas. FTLD-TDP cases adopt a more intermediate position and although primarily a cortical disorder, also exhibited random distributions of inclusions in some gyri.

Discussion

Comparison between the different disorders is complicated by three factors. First, different antibodies were used for the various tauopathies. However, no differences in density, type, or morphology of NCI have been observed between the tau antibodies used (Armstrong et al 2001). Second, there are different pathways of spread in the different disorders and the regions selected for study, mainly frontal and temporal, may not reflect the critical regions in all disorders. In AD, for example, the entorhinal cortex and PHG are affected early in the disease process, the pathology then spreading to affect the association areas of the brain and finally the primary sensory regions (De lacoste and White 1993). By contrast, in PiD, early stages of the disease are represented by loss of neurons in the fronto-insular cortex and anterior cingulate gyrus while the rest of the neocortex is affected much later in the disease. However, SFG, ITG, and PHG were studied in all disorders and are likely to be significantly affected by the relevant pathology. Third, different stages of the disease process are likely to be present within the various disorders. To attempt to address the effect of disease stage, the correlation between cluster size of inclusions and disease duration

and disease stage was investigated. However, the data provided little evidence that cluster sizes altered consistently during the disease process which could have been due to the small number of cases studied within each disease category.

The data suggest that all types of NCI, regardless of morphology or molecular composition, were frequently clustered in the cortex and, in a significant proportion of gyri, the clusters of NCI were regularly distributed parallel to the pia mater (Armstrong et al 2001). The frequency of this spatial pattern varied between disorders, being most frequent in NIFID and AD and least common in MSA. This spatial pattern includes disorders which have a prominent additional glial pathology, e.g., CBD (Armstrong et al 2009), PSP (Armstrong et al 2007), MSA (Armstrong et al 2006a), and FTLD-TDP (Armstrong et al 2010) or an additional molecular pathology in the form of beta-amyloidosis, as in AD (Armstrong 2009b). In addition, the PCA suggested the disorders could be divided into two groups: (1) AD, DLB, PiD, CBD, and NIFID which are characterised largely by regularly distributed clusters of inclusions, random distributions being rare and (2) PSP and MSA, in which random distributions of NCI were more frequent. FTLD-TDP occupies a somewhat intermediate position, NCI being essentially distributed in regularly distributed clusters but with a significant proportion of gyri exhibiting random distributions.

We suggest that the spatial patterns of the NCI is consistent with their development in association with the cells of origin of specific anatomical pathways (Armstrong 1993a, 2008, Armstrong et al 2002) and therefore could be the result from cell to cell transfer of pathogenic proteins (Goedert et al 2010, Steiner et al 2011). In cortical regions, the cells of origin of the cortico-cortical projections are clustered and occur in bands which are more or less regularly distributed along the cortex. Individual bands of cells traverse the cortical laminae and, in the primate brain, these bands of cells may vary in width from 400 - 500 μ m up to 800 - 1000 μ m depending on cortical region (Hiorns et al 1991, De Lacoste and White 1993). In a proportion of gyri, the width of the NCI clusters and their distribution along the cortex is consistent with an association with these pathways with two exceptions. Firstly, in some gyri, NCI occurred in significantly larger clusters, usually greater than 1600 μ m in diameter, and in some regions, small clusters of NCI were aggregated into larger 'superclusters'.

These results suggest that the smaller regularly distributed clusters of inclusions may ‘coalesce’ to form larger clusters as transfer and aggregation of pathogenic proteins proceeds (Armstrong 1993a). Secondly, NCI are randomly distributed in some gyri especially in PSP and MSA and to some extent in FTLD-TDP. Random distributions of inclusions are usually the result of low density (Armstrong et al 2001) which is more likely to be the case in the cortex in PSP and MSA; primarily subcortical disorders (Armstrong 2006a, 2007). In contrast, FTLD-TDP is predominantly a cortical disorder but the NCI also exhibit random distributions in a significant proportion of gyri. Multiple TDP-immunoreactive pathologies including NCI, glial inclusions (GI), neuronal intranuclear inclusions (NII), and dystrophic neuritis (DN), however, are characteristic of FTLD-TDP and the density of NCI alone can be quite low especially in some subtypes of the disease (Armstrong et al 2010).

Despite similarities in spatial pattern, there were differences in cluster sizes of NCI suggesting variation in the degree to which specific cortical columns are affected in the different diseases. Hence, the largest clusters were observed in AD, PiD, DLB, and CBD and the smallest in PSP, MSA, FTLD-TDP, and NIFID. In MSA and PSP, the small cluster size could be attributable to the largely subcortical pathology, cortical degeneration occurring later in the disease process (Bigio et al 1999, Armstrong 2006a, 2007). In FTLD-TDP and NIFID-FUS, however, clusters of NCI were usually 50 - 200 μ m in diameter, significantly smaller than the estimated diameter of the cell columns of the cortico-cortical projections. Hence, a more localised pattern of cortical degeneration appears to be present in NIFID and FTLD-TDP and there could be additional molecular pathologies that may contribute to cortical degeneration in these two disorders.

Hence, NCI characterised by different molecular pathologies appear to be associated with similar spatial patterns, viz., the regularly distributed cluster of inclusions. In each disorder, there is the formation of a unique, abnormally aggregated, or misfolded protein which either causes neurodegeneration of specific anatomical pathways or results in a cellular response designed to sequester proteins into less harmful aggregates (Forman et al 2004, Armstrong 2009b, Armstrong et al 2008). A variety of mechanisms have been proposed by which misfolded proteins can directly affect cellular homeostasis including disruption of the ubiquitin degradation system, axonal

transport, synaptic function, and protein sequestration (Forman et al 2004). Of particular interest is whether the spatial patterns reflect cell to cell transfer of pathogenic proteins as suggested by Steiner et al. (2011). Proteins such as tau and α -synuclein can exit host cells, transfer between cells, gain access to new cells, and create pathology within these cells (Steiner et al 2011). For example, α -synuclein taken up from the extracellular space can induce aggregation of other α -synuclein proteins in recipient cells. By analogy with the scrapie form of prion protein (PrP^{Sc}), nucleation or seeding activity of α -synuclein may result in a core of an NCI of transferred α -synuclein surrounded by additional layers of cytoplasmic α -synuclein contributed by the host cell. It is also possible that TDP-43 and FUS may have these properties and therefore, that cell to cell transfer may be a common mechanism determining cortical degeneration in a range of diseases.

In conclusion, neurodegenerative disorders characterised by NCI expressing different molecular pathologies and varying in morphology exhibit similar spatial patterns in the cerebral cortex, consistent with an association with specific anatomical pathways. The data provide some support for the hypothesis of cell to cell transfer of pathogenic proteins and that there are common patterns of cortical neurodegeneration occurring across the different disorders. It would be interesting to follow the spatial pattern of inclusions along the anatomical pathways in the various disorders to test more rigorously the hypothesis of cell to cell transport. Different disorders may be amenable to similar interventions, e.g., immunotherapy which targets extracellular pathogenic proteins could lead to their removal thus preventing or slowing cell to cell propagation (Villolslada et al 2008).

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Table 1. Summary of demographic details, diagnostic criteria, immunohistochemistry, and brain areas studied in the different disorders.

<u>Disorder</u>	<u>N</u>	<u>Age</u>	<u>M:F</u>	<u>IHC</u>	<u>Diagnostic criteria</u>	<u>Regions studied</u>
AD	6	78 (9.2)	3:3	AT8 (tau)	NINCDS/ ADRDA/ CERAD	SFG, SPL, ITG, PHG VC
PiD	10	65.3 (11.3)	7:3	TP70 (tau)	Cairns et al (2007)	SFG, ITG, PHG
CBD	12	64.7 (9.07)	8:4	TP007 (tau)	NIH-ORD	SFG, SPL, ITG, PHG
PSP	8	73.4 (7.4)	4:4	TP70 (tau)	NINDS- SPSP	SFG, ITG, PHG
DLB	12	73.8 (7.2)	12:0	α 116	CDLB (α -synuclein)	SFG, ITG, PHG
MSA	10	66.5 (8.51)	7:3	α 116	MCC (α -synuclein)	SFG, ITG, PHG
NIFID	10	45.3 (12.1)	7:3	FUS	Cairns et al (2007)	SFG, ITG, PHG
FTLD- TDP	15	69.6 (8.50)	7:2	TDP-43	Cairns et al (2007)	SFG, ITG, PHG

Abbreviations: Alzheimer's disease (AD), Pick's disease (PiD), corticobasal degeneration (CBD), progressive supranuclear palsy (PSP), dementia with Lewy bodies (DLB), multiple system atrophy (MSA), neuronal intermediate filament inclusion disease (NIFID), frontotemporal lobar degeneration with TDP-43

proteinopathy (FTLD-TDP); FUS = 'fused in sarcoma' TDP-43 = transactive response (TAR) DNA-binding protein, N = Number of cases studied, IHC = Immunohistochemistry, M = Male, F = Female, SFG = Superior frontal gyrus (B8), SPL = Superior parietal lobule (B7a/7b), ITG = Inferior temporal gyrus (B22), PHG = parahippocampal gyrus (B28), VC = Visual cortex (B17). Diagnostic criteria: 'National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer Disease and Related Disorders Association' (NINCDS/ADRDA) (Tierney et al 1988), 'Consortium to Establish a Registry of Alzheimer Disease' (CERAD) criteria (Mirra et al 1991); National Institute of Health-Office of rare disorders (NIH-ORD); National Institute of Neurological Disorders and Stroke (NINDS) and the Society of PSP (SPSP) (NINDS-SPSP) (Hauw et al 1994, Litvan et al 1996); 'Consortium on Dementia with Lewy bodies' (CDLB)' (McKeith et al 1996); 'Minneapolis Consensus Criteria (MCC) (Gilman et al 1998); Diagnostic criteria for PiD, NIFID and FTLD-TDP according to Cairns et al (2007).

Table 2. Frequency of the different types of spatial pattern (R = Random, U/RG = Uniform or Regular, RGC = in regularly distributed clusters, 50 – 1600µm in diameter, LC = in large clusters, ≥1600µm in diameter) exhibited by neuronal cytoplasmic inclusions (NCI) in the upper (U) and lower (L) cortex of various neurodegenerative disorders: Alzheimer's disease (AD), Pick's disease (PiD), corticobasal degeneration (CBD), progressive supranuclear palsy (PSP), dementia with Lewy bodies (DLB), multiple system atrophy (MSA), neuronal intermediate filament inclusion disease (NIFID), frontotemporal lobar degeneration with TDP-43 proteinopathy (FTLD-TDP), NFT = neurofibrillary tangles, PB = Pick bodies, LB = Lewy bodies. N = total number of regions analysed for each disorder.

Frequency of spatial pattern

<u>Disorder</u>	<u>NCI</u>	<u>LM</u>	<u>N</u>	<u>R</u>	<u>U/RG</u>	<u>RGC</u>	<u>LC</u>	<u>%RGC</u>
AD	NFT	U	17	1	0	12	4	
		L	13	0	0	10	3	
		Total	30	1	0	22	7	73
PiD	PB	U	29	1	0	16	12	
		L	19	0	0	11	8	
		Total	48	1	0	27	20	53
CBD	NCI	U	43	2	1	24	16	
		L	33	0	1	24	8	
		Total	76	2	2	48	24	63
PSP	NFT	U	11	6	0	5	0	
		L	12	5	0	5	2	
		Total	23	11	0	10	2	43
DLB	LB	U	38	1	1	23	13	
		L	32	1	0	20	11	
		Total	70	2	1	43	24	61
MSA	NCI	U	6	3	0	2	1	
		L	3	2	0	1	0	
		Total	9	5	0	3	1	33
NIFID	NCI	U	27	4	1	20	2	

		L	26	3	2	19	2	
		Total	53	7	3	39	4	74
FTLD-TDP	NCI	U	30	9	0	20	1	
		L	19	9	1	7	2	
		Total	49	18	1	27	3	55

Chi-square (χ^2) contingency tests comparing totals of upper and lower cortex:

Comparing all groups: $\chi^2 = 107.78$ (21DF, $P < 0.001$)

Comparing tauopathies and synucleinopathies: $\chi^2 = 0.16$ (3DF, $P > 0.05$)

Comparing tauopathies: $\chi^2 = 55.27$ (9DF, $P < 0.001$)

Comparing 3R tauopathies with 4R tauopathies: $\chi^2 = 5.94$ (3DF, $P > 0.05$)

Comparing synucleinopathies: $\chi^2 = 27.36$ (3DF, $P < 0.001$)

Comparing PiD and CBD: $\chi^2 = 2.52$ (3DF, $P > 0.05$)

Comparing AD and PSP: $\chi^2 = 9.47$ (2DF, $P < 0.01$)

Comparing tauopathies and NIFID: $\chi^2 = 13.96$ (3DF, $P < 0.001$)

Comparing tauopathies and FTLD-TDP: $\chi^2 = 28.87$ (3DF, $P < 0.001$)

Comparing synucleinopathies and NIFID: $\chi^2 = 12.13$ (3DF, $P < 0.001$)

Comparing synucleinopathies and FTLD-TDP: $\chi^2 = 21.20$ (3DF, $P < 0.001$)

Comparing different morphologies: $\chi^2 = 7.34$ (3DF, $P > 0.05$)

Table 3. Frequency of brain regions in which regularly distributed clusters (RGC) of neuronal cytoplasmic inclusions (NCI) were either in the size range 400 – 800µm or exhibited a more complex clustering pattern in which small clusters were aggregated to form larger clusters in various neurodegenerative disorders: Alzheimer's disease (AD), Pick's disease (PiD), corticobasal degeneration (CBD), progressive supranuclear palsy (PSP), dementia with Lewy bodies (DLB), multiple system atrophy (MSA), neuronal intermediate filament inclusion disease (NIFID), frontotemporal lobar degeneration with TDP-43 proteinopathy (FTLD-TDP), NFT = neurofibrillary tangles, PB = Pick bodies, LB = Lewy bodies.

Frequency of spatial pattern

<u>Disorder</u>	<u>NCI</u>	<u>RGC (400-800µm)</u>	<u>Clusters/Superclusters</u>
AD	NFT	6	4
PiD	PB	15	2
CBD	NCI	24	9
PSP	NFT	2	0
DLB	LB	14	6
MSA	NCI	0	0
NIFID	NCI	11	1
FTLD-TDP	NCI	5	6

Legends to figures

Fig 1. Morphometric method of sampling the cerebral cortex in the various neurodegenerative disorders. Inclusions were counted along two strips of tissue parallel to the pia mater (1600 to 3200 μm in length) using 50 x 250 μm contiguous sample fields (X_1 to X_5 etc). The sample fields were located both in the upper and lower cortical laminae and extend along the gyrus from sulcus to sulcus; the short edge of the field being aligned with guidelines marked on the slide.

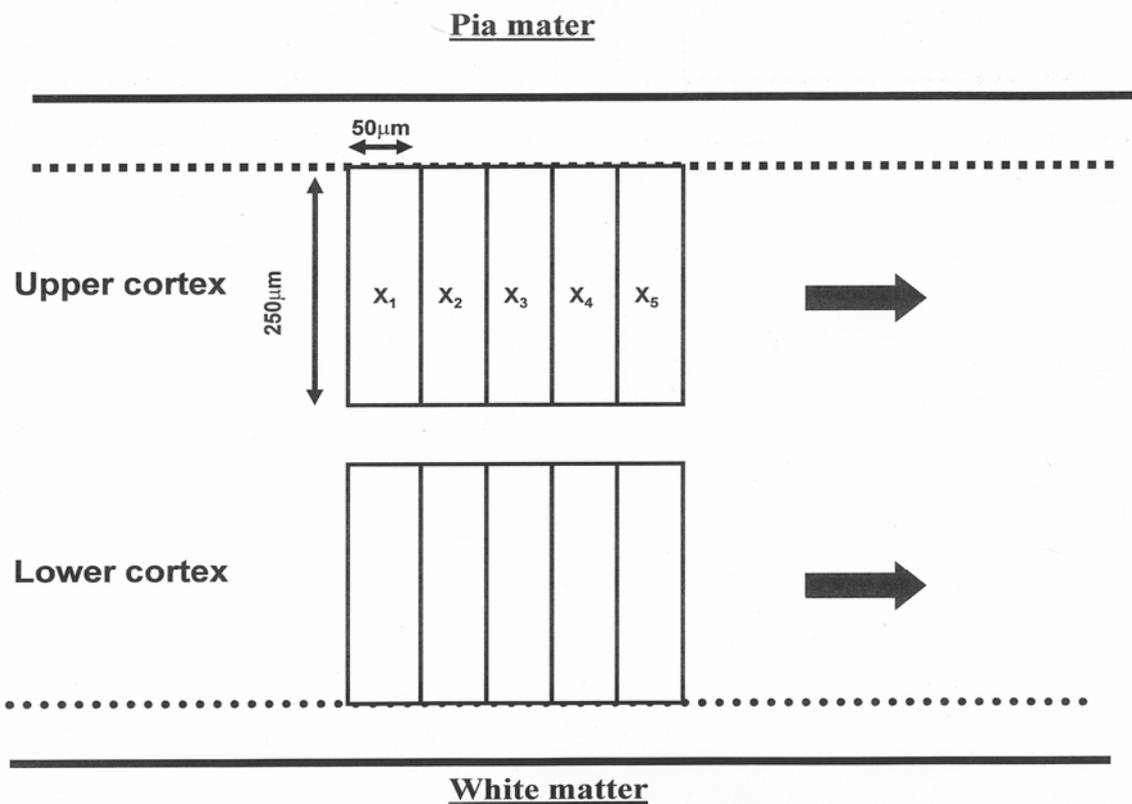


Fig 2. A random distribution of tau-immunoreactive neuronal inclusions in the tauopathy corticobasal degeneration (CBD). (tau immunohistochemistry, TP007 antibody, haematoxylin, Bar = 80µm)

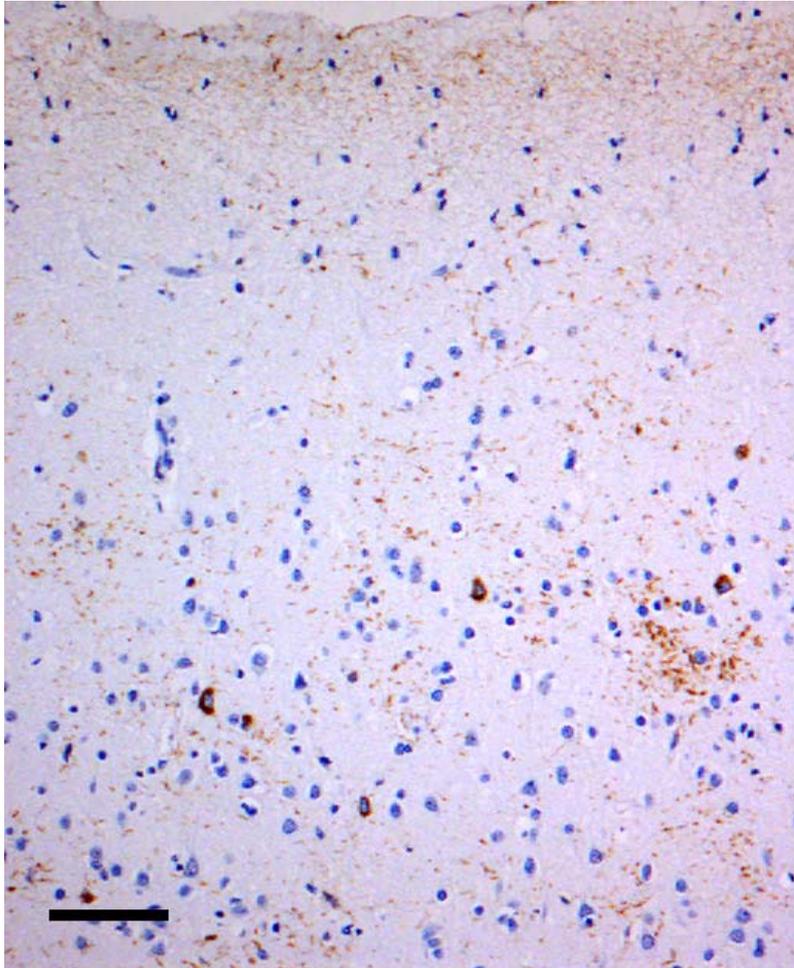


Fig 3. A small cluster of Lewy bodies (LB), approximately 70 μ m in diameter, in the synucleinopathy dementia with Lewy bodies (DLB) (α -synuclein immunohistochemistry, α 116 antibody, haematoxylin, Bar = 25 μ m)

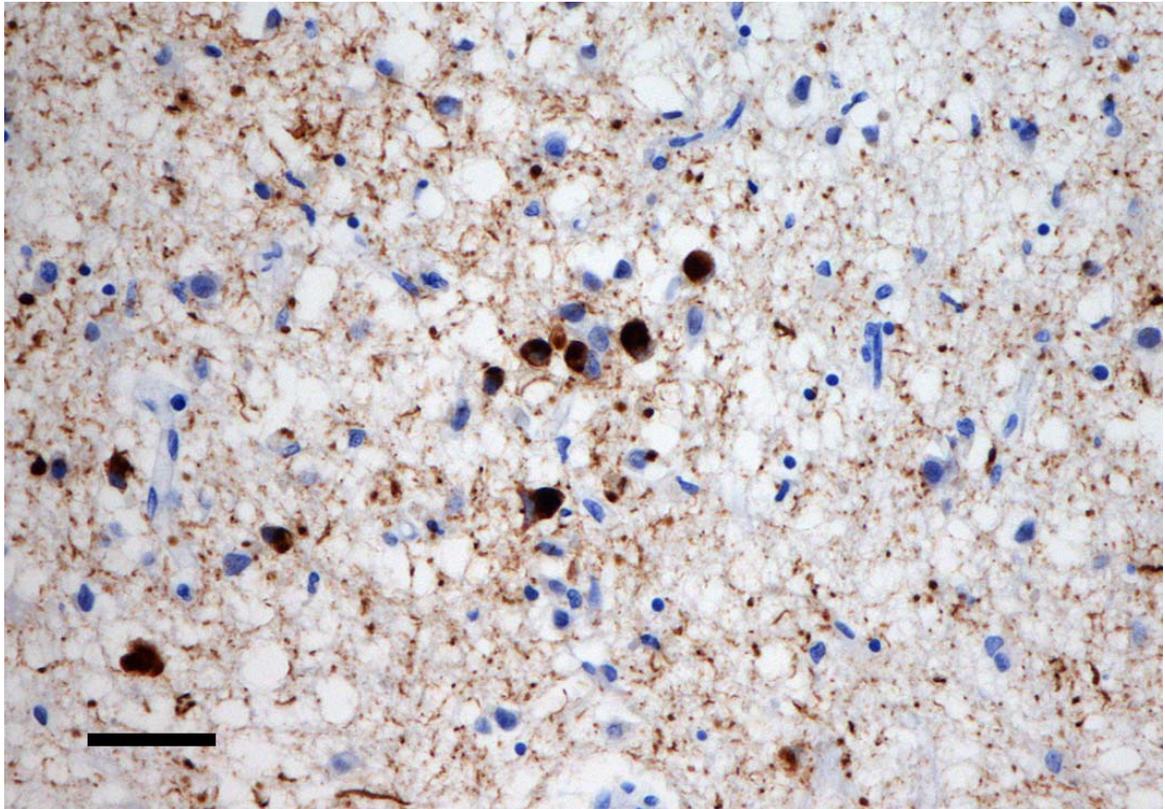


Fig 4. A larger cluster of neuronal cytoplasmic inclusions (NCI) in neuronal intermediate filament inclusion disease (NIFID) NIFID (FUS immunohistochemistry, haematoxylin, Bar = 25µm)

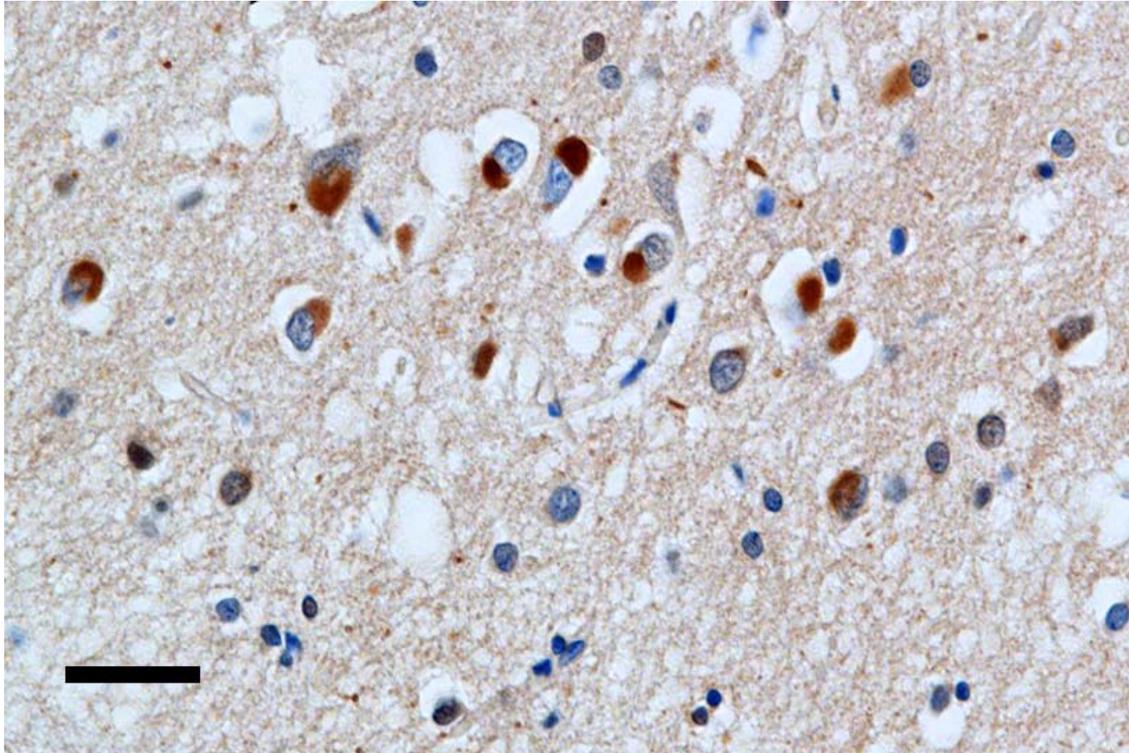


Fig 5. Pattern analysis plots showing examples of the spatial patterns exhibited by neuronal cytoplasmic inclusions (NCI) in the inferior temporal gyrus (ITG): A comparison of Lewy bodies (LB) in dementia with Lewy bodies (DLB) and Pick bodies (PB) in Pick's disease (PiD). Significance of V/M peaks, * $P < 0.05$, ** $P < 0.01$.

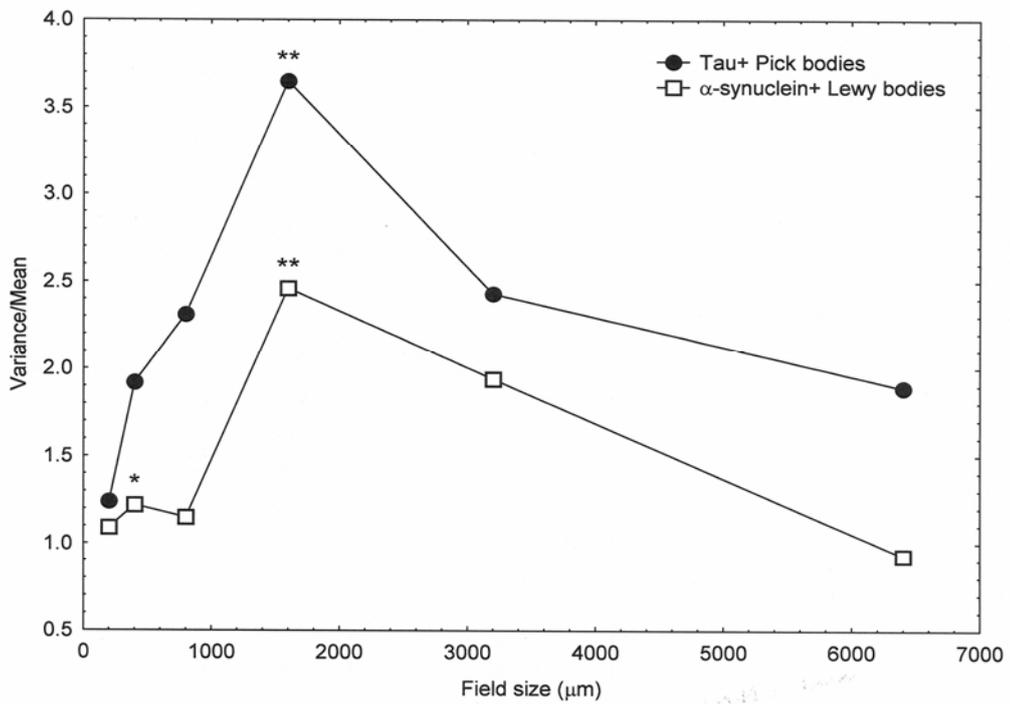


Fig 6. Mean cluster size of the neuronal cytoplasmic inclusions (NCI) in the cortex of Alzheimer's disease (AD), Pick's disease (PiD), corticobasal degeneration (CBD), progressive supranuclear palsy (PSP), dementia with Lewy bodies (DLB), multiple system atrophy (MSA), neuronal intermediate filament inclusion disease (NIFID), frontotemporal lobar degeneration with TDP-43 proteinopathy (FTLD-TDP). Analysis of variance (ANOVA) (one-way with Scheffé's *post-hoc* tests): Cluster size $F = 14.73$ ($P < 0.001$); Significant differences between groups: AD > PSP, NIFID, FTLD; PiD > PSP, NIFID, FTLD; CBD > NIFID, FTLD; DLB > PSP.

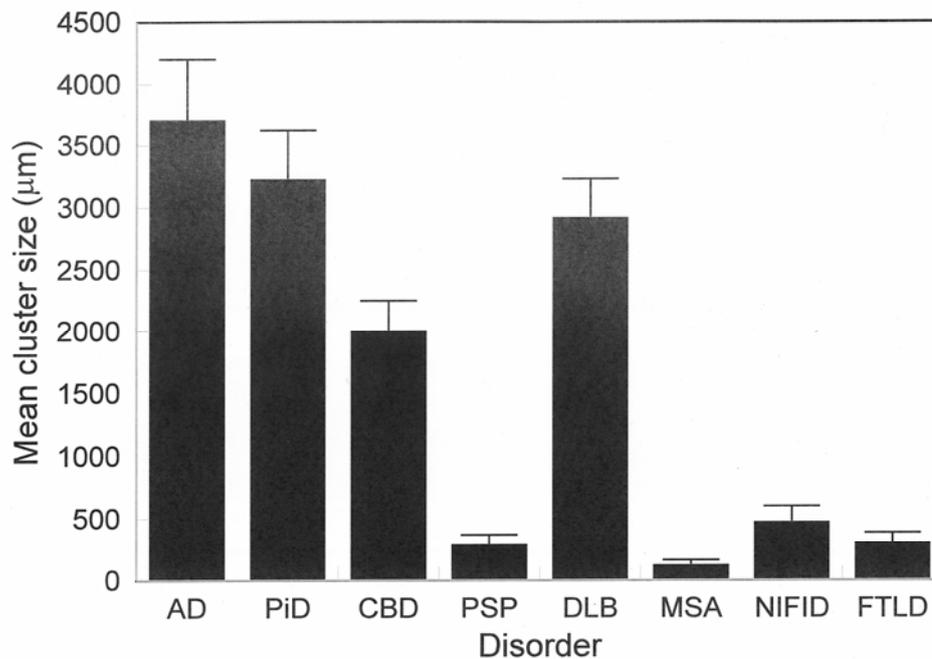


Fig 7. A principal components analysis (PCA) of the neurodegenerative diseases based on the frequency of different types of spatial pattern: Alzheimer's disease (AD), Pick's disease (PiD), corticobasal degeneration (C), progressive supranuclear palsy (PSP), dementia with Lewy bodies (D), multiple system atrophy (MSA), neuronal intermediate filament inclusion disease (NIFID), frontotemporal lobar degeneration with TDP-43 proteinopathy (FTLD-TDP).

