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ANALYSIS OF β -AMYLOID ($A\beta$) DEPOSITION IN THE TEMPORAL LOBE IN ALZHEIMER'S DISEASE USING FOURIER (SPECTRAL) ANALYSIS

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Abstract

Aim—To determine the spatial pattern of β -amyloid ($A\beta$) deposition throughout the temporal lobe in Alzheimer's disease (AD).

Methods—Sections of the complete temporal lobe from six cases of sporadic AD were immunolabelled with antibody against $A\beta$. Fourier (spectral) analysis was used to identify sinusoidal patterns in the fluctuation of $A\beta$ deposition in a direction parallel to the pia mater or alveus.

Results—Significant sinusoidal fluctuations in density were evident in 81/99 (82%) analyses. In 64% of analyses, two frequency components were present with density peaks of $A\beta$ deposits repeating every 500–1000 μ m and at distances greater than 1000 μ m. In 25% of analyses, three or more frequency components were present. The estimated period or wavelength (number of sample units to complete one full cycle) of the first and second frequency components did not vary significantly between gyri of the temporal lobe, but there was evidence that the fluctuations of the classic deposits had longer periods than the diffuse and primitive deposits.

Conclusions—(i) $A\beta$ deposits exhibit complex sinusoidal fluctuations in density in the temporal lobe in AD, (ii) fluctuations in $A\beta$ deposition may reflect the formation of $A\beta$ deposits in relation to the modular and vascular structure of the cortex, and (iii) Fourier analysis may be a useful statistical method for studying the patterns of $A\beta$ deposition both in AD and in transgenic models of disease.

Keywords

Alzheimer's disease; Fourier (spectral) analysis; β -amyloid ($A\beta$) deposits; Spatial pattern; Spectral density; Wavelength

Introduction

β -amyloid ($A\beta$) deposition in the form of diffuse ('pre-amyloid'), primitive, and classic ('dense-cored') deposits is a hallmark pathological feature of Alzheimer's disease (AD) [1–3]. A variety of $A\beta$ peptides are present within $A\beta$ deposits and are formed as a result of secretase cleavage of the membrane-bound glycoprotein amyloid precursor protein (APP)

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[2–4]. The most common of these peptides is A β ₄₂ found largely in discrete A β deposits, whereas the more soluble A β ₄₀ is also found in association with blood vessels [5,6] and may develop later in the disease [7]. In addition, A β deposits have a variety of ‘secondary’ molecular constituents [8] including acute-phase proteins such as α -antichymotrypsin and α ₂-macroglobulin [9], intercellular adhesion molecule 1 (ICAM1) [9], apolipoprotein E (Apo E) [10] and D (Apo D) [11], the heterodimeric glycoprotein clusterin, vitronectin, the complement proteins C1q, C4, and C3 [12], blood proteins such as amyloid-P, and the sulphated glycosaminoglycans such as heparin sulphate proteoglycan (HSPG).

In the cortex and hippocampus of patients with AD, there are regularly repeating patterns of A β deposition in a direction parallel to the tissue boundary [13]. These variations in density may reflect the degeneration of specific anatomical projections and studies of the spatial patterns of A β deposits have been useful in elucidating the relationship between pathology and neuroanatomy [14–16]. To study the spatial patterns of A β deposits, the densities of deposits are recorded in ‘transects’ of contiguous sample fields or plots aligned parallel with the tissue boundary [17]. The frequency distribution of A β deposit density in the plots is compared with that predicted by the Poisson distribution; a variance/mean ratio greater than unity indicating clustering of deposits [18,19]. Counts of deposits in adjacent sample fields are then added together successively to provide data for larger field sizes thus enabling the cluster size and the distribution of the clusters parallel to the tissue boundary to be investigated.

The Poisson method of studying spatial patterns has a number of limitations [18]. First, the method can only be used to analyse counts of deposits and not alternative measures of abundance such as ‘coverage’ or ‘load’ [17]. Second, a linear trend in abundance of deposits parallel to the tissue boundary, or the presence of a single large cluster of deposits occupying a significant region of a gyrus, make it more difficult to detect smaller-scale clustering patterns. Third, the method does not estimate the cluster size of the deposits accurately enough to relate spatial patterns to neuroanatomical features. Fourth, A β deposits are not usually distributed in discrete clusters but show a more continuous fluctuating pattern of abundance along cortical gyri parallel to the pia mater and fluctuations repeating at different scales may be present.

Bruce et al [19] were the first to suggest that Fourier analysis might be used to analyse spatial fluctuations in A β deposition especially across the cortex from pia mater to white matter. Fourier analysis is an attempt to understand the pattern of fluctuations of a quantity in space or time (known as the ‘signal’) by breaking it down into simpler sine waves which can then be used to resynthesise the original signal. Hence, the original signal can be thought of as comprising sine waves of different frequency, the sine waves being easier to study and interpret than the original more complex signal. The method has been applied in many fields including physics, mathematics, imaging, probability theory, and acoustics. In the present neuropathological context, the complex spatial fluctuations in A β deposition constitute the ‘signal’ and the analysis attempts to find the sine waves which comprise the frequency components of the signal. The frequency components may then be able to be interpreted in relation to the underlying neuroanatomical structure of the cerebral cortex.

In the present study, Fourier (spectral) analysis was applied to variations in A β deposition along the tissue parallel to the pia mater or alveus in regions of the temporal lobe in AD and had the following objectives: 1) to determine whether there were significant sinusoidal fluctuations in the distribution of the A β deposits, 2) if significant fluctuations were present, to determine the ‘periods’ or wavelengths of the various frequency components, 3) to determine whether recurring patterns of A β deposition could be related to neuroanatomy and

especially to the modular structure of the cerebral cortex and 4) the significance of the results to the pathology of AD.

Materials and methods

Cases

Six cases of sporadic AD and six elderly non-demented control cases (details in Table 1) were obtained from the Brain Bank, Department of Neuropathology, Institute of Psychiatry, King's College, London. Informed consent was given for the removal of all tissue and followed the principles embodied in the 1964 Helsinki declaration (as modified Edinburgh, 2000). Post-mortem delay was less than 20 hours in each case. The control cases had no neurological or psychiatric histories and were matched as closely as possible for age to the AD cases. The AD cases were clinically assessed and all fulfilled the 'National Institute of Neurological and Communicative Disorders and Stroke and Alzheimer's Disease and Related Disorders Association' (NINCDS/ADRDA) criteria for probable AD [20]. The histological diagnosis of AD was established by the presence of widespread neocortical senile plaques (SP) consistent with the 'Consortium to Establish a Registry of Alzheimer's Disease' (CERAD) criteria [21]. In addition, neurofibrillary tangles (NFT) were abundant in the cerebral cortex and hippocampus of each AD case. Apo E genotype of the AD cases was either 3/4 or 4/4 and the cases represented Braak stages IV, V, or VI [22]. Control cases had an apo E genotype of 3/3 or 3/4 and represented Braak stages I to III. Capillary amyloid angiopathy (CAA) is present in a proportion of AD cases and may be related to apo E genotype [23]. Although small amounts of CAA were observed in some larger vessels, none of the cases had sufficient CAA for a diagnosis of AD with CAA (AD/CAA) and it was not possible to study the relationship between A β deposition and the extent of CAA.

Tissue preparation

Coronal sections of the complete temporal lobe were taken at the level of the lateral geniculate body from each case. Sections, 7 μ m in thickness, were stained with a rabbit polyclonal antibody (Gift of Prof. B.H. Anderton) raised against the 12–28 amino acid sequence of the A β protein [24] to reveal the deposits. The antibody was used at a dilution of 1 in 1200 and incubated at 4° overnight. Sections were pretreated with 98% formic acid for 6 minutes to enhance immunoreactivity. A β was visualised using the streptavidin-biotin horseradish peroxidase procedure with diaminobenzidine as the chromogen. The three most common morphological subtypes of A β deposit were identified in the sections using previously published criteria (Fig 1) [2,3]. Hence, diffuse deposits were 10–200 μ m in diameter, irregular in shape with diffuse boundaries, and lightly stained. Large confluent patches of A β immunostaining, which could be a variant of diffuse deposit, were not quantified. Primitive deposits, were 20–60 μ m, well demarcated, more symmetrical in shape, and strongly stained. Classic deposits were 20–100 μ m, had a distinct central 'core' surrounded by a 'corona' of dystrophic neurites. In a thin section, one does not see the projection of the whole A β deposit but a sectional profile, the shape and size of which depends on where the deposit is cut. In addition, deposits are hit by sectioning planes with a probability proportional to their tangent diameter orthogonal to the section. Hence, large deposits are cut by many planes while small deposits are hit more rarely. The sectional profiles of deposits revealed in two dimensional sections are therefore not a random sample of such deposits in a volume of tissue and the parameters estimated from analysing their planar distribution are not valid estimates of their true distribution in three dimensions. Hence, deposits of A β less than 10 μ m, which could be identified as small diffuse or primitive deposits, may actually represent the tissue section cutting tangentially through the corona of larger classic deposits. As a consequence, deposits less than 10 μ m cannot be identified to subtype and were not counted.

Morphometric methods

The distribution of the A β deposits was studied in the superior temporal gyrus (STG), middle temporal gyrus (MTG), inferior temporal gyrus (ITG), lateral occipitotemporal gyrus (LOT), parahippocampal gyrus (PHG), and the CA sectors of the hippocampus. In cortical gyri, A β deposition was studied along the entire length of the gyrus from sulcus to sulcus using 1000 $\times$ 200 μ m contiguous sample fields, the short dimension of the plot being aligned with the surface of the pia mater. Hence, the plot included laminae I, II, and most of III; the region containing the highest densities of A β deposits in AD [25]. A micrometer grid with grid lines at intervals of 10 μ m was used as the sample field. Between 64 and 128 contiguous sample fields were usually required to sample each gyrus. In the hippocampus, the sample fields were aligned with the alveus to sample sectors CA1 to CA3. Sampling was continued into sector CA4 using a guideline marked on the slide and which ceased approximately 400 μ m from the DG granule cell layer. The number of diffuse, primitive, and classic A β deposits was counted in each sample field.

Data analysis

The data were analysed by single spectrum (Fourier) analysis [26,27] using STATISTICA software (Statsoft Inc., 2300 East 14th St, Tulsa, Ok, 74104, USA). Spectrum analysis explores whether there are cyclical patterns in data either in space or time and the specific objective is to 'decompose' a complex sequence of contiguous data into a few underlying sinusoidal functions. The analysis was applied to the diffuse, primitive, and classic A β deposits in each gyrus and hippocampus from each AD case (a total of 108 analyses) and control case (a total of 30 analyses). First, variations in the density of A β deposits along each gyrus and CA region were examined for a linear trend. If such a trend was present it may obscure smaller-scale patterns and the data were 'corrected' to remove the effect of the linearity. Second, the data from each gyrus were tested to determine whether there were any statistically significant sinusoidal fluctuations present. If the fluctuations in density parallel to the tissue boundary were simply random 'noise', then the raw periodogram values (spectral densities) would follow an exponential distribution. Hence, the exponential distribution was fitted to the frequency distribution of periodogram values and goodness-of-fit tested using the Fisher-Kappa statistic and the Kolmogorov-Smirnov (KS) test. Third, if the data deviated from an exponential distribution, significant recurring sinusoidal patterns were present and the periodogram values were 'smoothed' to reduce the effect of measurement noise. The spectral density plot was then examined for significant peaks which indicate the 'periods' of the recurring fluctuations, i.e., the number of adjacent 200 μ m plots necessary to complete one full cycle.

Results

An example of Fourier analysis applied to the diffuse A β deposits in the MTG of a single case (Case C) is shown in Figs 2–4. Fig 2 shows the variations in density of the diffuse deposits along the gyrus and reveals a complex fluctuating pattern. Fig 3 shows the frequency distribution of the periodogram (spectrogram) values, a distribution that deviates significantly from the exponential distribution (Fisher-Kappa = 8.345, KS = 0.43, $P < 0.001$) indicating the presence of sinusoidal fluctuations. Fig 4 is a plot of the spectral density at each period. Three significant peaks are evident suggesting the presence of three frequency components: 1) at a period of 2.62 units representing a complete A β deposit density cycle every 524 μ m (i.e., $2.62 \times$ basic plot size of 200 μ m), 2) at a period of 12 units representing fluctuation of deposits on a larger scale with cycles recurring every 2400 μ m, and 3) maximum spectral density at a period of 42 units representing an even larger scale of fluctuation repeating every 8400 μ m.

A summary of the results from all regions and cases is shown in Table 2. Of the total of 108 analyses from the AD cases, 9 had insufficient A β deposits to analyse spectrally. Of the remaining 99 analysis, 25/99 (25.3%) were corrected for a linear trend: 18/99 (18.2%) exhibited no statistically significant recurring sinusoidal pattern, while in 81/99 (81.8%) there was evidence of significant recurring fluctuations of A β density of at least one frequency. Of the data exhibiting significant frequency components, 9/81 (11%) exhibited a single frequency component (periods <1000 μ m in four analyses and >1000 μ m in five analyses), 52/81 (64%) exhibited two frequency components, and in 51 of these analyses, the first frequency component had a period of <1000 μ m and the second >1000 μ m. In 20/81 (25%) of analyses, three or more frequency components were present. In control cases, no A β deposits were observed in the STG, ITG, or the CA sectors of the hippocampus but A β deposits were observed in the LOT and PHG. Data from the control cases suggest that of a total of 30 analyses, 3 had insufficient A β deposits to analyse spectrally. Of the remaining 27 analysis, 3 had to be corrected for a linear trend: 12/27 (44%) exhibited no statistically significant recurring sinusoidal pattern, while in 15/27 (55%) there was evidence of significant recurring fluctuations of A β density of at least one frequency. Of the data exhibiting significant frequency components, 1/15 (7%) exhibited a single frequency component, 4/15 (27%) exhibited two frequency components, and in 10/15(67%) of analyses, three or more frequency components were present.

The summary statistics for the distribution of the periods of the various frequency components in the AD and control cases are shown in Table 3. In AD, the period of the first frequency component had a mean of 910.70 μ m (range 392–7800 μ m), the second period a mean of 3534 μ m (range 600–16800 μ m), and where three or more frequency components were present, they had an overall mean period of 5919 μ m (range 1600–14000 μ m). The distribution of the periods of the first frequency component was positively skewed (Skew = 2.60, Standard error = 0.18) and had a median and mode of 600 μ m. In the control cases, the period of the first frequency component had a mean of 140 μ m (range 100–300 μ m), the second period a mean of 1081 μ m (range 320–3700 μ m), and where three or more frequency components were present, they had an overall mean period of 2109 μ m (range 450–3750 μ m).

There were insufficient data to compare deposit subtypes and brain regions in control cases. The average period of the first and second frequency components in each brain region in AD is shown in Table 4. The period of the first and second frequency component did not differ significantly between the different regions of the temporal lobe. However, the average period of the first frequency component was significantly less for the diffuse and primitive A β deposits than for the classic deposits.

Discussion

The first objective was to determine whether there were significant cyclic fluctuations in A β deposition in a direction parallel to the pia mater or alveus. In the majority of regions analysed in the AD cases, there were significant recurring patterns of A β deposition in the upper cortical laminae and in the hippocampus. These results are in agreement with previous studies employing the Poisson distribution [18,28,29] in suggesting the presence of regularly repeating patterns of A β deposition in AD [16,18]. A β deposition also occurred in the control cases but with a more restricted distribution than in AD being found only in the LOT and PHG. Fourier analysis of these areas showed some significant differences compared with AD. First, there were more regions in control brain in which there were no significant fluctuations in A β deposition than in AD. Second, where significant cyclic fluctuations were present they had significantly smaller periods than in AD, i.e., the A β deposits fluctuated over much smaller scales than in AD.

The second objective was to determine the periods of the significant frequency components. The commonest spatial pattern observed was a small-scale fluctuation in A β density, a complete cycle repeating with a mean distance of 901 μ m (range 392–7800 μ m), and a larger-scale pattern repeating at distances greater than 1000 μ m (mean 3534 μ m, range 600–16800 μ m). In some regions, a more complex pattern was present with three or more frequency components, the largest-scale fluctuations having a mean period of 5919 μ m (range 1600–14000 μ m). The distribution of the periods of the smallest frequency component was positively skewed and had a median size of 600 μ m. These results are also in agreement with previous studies of the temporal lobe in AD [18,28,29] in suggesting clustering of A β deposits, the clusters being 500–1000 μ m in diameter [16,18].

The third objective was to determine whether the variations in A β deposition could be related to neuroanatomy. A major feature of the anatomical structure of the cerebral cortex is the replicated local neural circuit (represented by ‘columns’ or ‘modules’) [30]. The diameter of individual cortical modules varies between 500–1000 μ m depending on region and there are specific connections maintained between ordered sets of columns [30,31]. First, there is a reciprocal projection between a cortical area and a dorsal thalamic nucleus. Second, there are inputs to the cortex from generalised regulatory systems, e.g., from the basal forebrain and monoaminergic nuclei of the brain stem. As a consequence, the cortex is interlaced by clusters of fine noradrenergic fibres which repeat at 30–40 μ m intervals. Third, homologous neocortical areas, with the exception of the striate cortex and primary somatosensory cortex, are reciprocally linked in both hemispheres via commissures; both the commissural and ipsilateral association fibres terminating in vertically oriented columns 200–500 μ m in width, alternating in a regular sequence with zones of comparable width free of such connections. Fourth, within a hemisphere, different regions are connected by fibres of the short and long cortico-cortical projections [15,31]. The cells of origin of these projections are also clustered and occur in bands approximately 500–1000 μ m in width.

Using a basic plot width of 200 μ m, it is not possible to determine frequency components repeating at intervals smaller than this size. Hence, we could not determine whether A β deposition was related to the generalized regulatory systems of the cortex. The smallest frequency component observed had a period consistently between 500 and 1000 μ m and a median dimension of 600 μ m. These dimensions suggest a spatial relationship between the A β deposits and the primary modules of the cortex postulated in previous studies [16,18]. Those gyri in which the period was significantly less than 500 μ m could represent the association of A β deposition with the commissural projections and those within the range 500–1000 μ m with the short and long cortico-cortical projections. In the majority of gyri, more than one spatial frequency component was present, the most common spatial pattern representing fluctuations of A β deposits repeating every 400–1000 μ m and at distances greater than 1000 μ m. In the cerebral cortex, adjacent columns are organized into larger functional units [30] and the larger-scale fluctuations may represent A β deposition in relation to these groupings of modules. In addition, deposition of A β could be a dynamic process and there may be coalescence of the smaller clusters of A β deposits to form much larger aggregations as the disease develops [18]. In approximately 25% of regions, there was a distinct linear trend in A β deposition parallel to the tissue boundary which may represent fluctuations in A β deposition on a very large scale perhaps reflecting the vulnerability of whole or adjacent groups of gyri. Sinusoidal variations in A β deposition were not confined to the cerebral cortex and were observed within the CA sectors of the hippocampus. These fluctuations may also reflect underlying neuroanatomy and specifically the modular structure of the alvear pathway and perforant path.

The pattern of fluctuation of the classic A β deposits was distinctly different to that of the diffuse and primitive deposits, being distributed in cycles of significantly longer period. The

classic-type of A β deposit is the only subtype to show a consistent spatial relationship with the cerebral microcirculation [29,32,33]. In the cerebral cortex, the larger arterioles penetrate the pia mater at intervals and then extend vertically through the laminae before reaching a maximum density in cortical lamina IV [34]. Hence, the larger diameter arterioles are often distributed in a regular pattern in the upper cortical laminae parallel to the pia mater and with periods greater than those of the repeating cortical modules [29]. Hence, regularity in the distribution of blood vessels may be an additional factor determining the sinusoidal fluctuations of the classic deposits.

The fourth objective was to assess the significance of the results to the pathology of AD as a whole. A β deposition also occurs in normal aging with a topography closely matching that of AD [35]. Of the types of senile plaque (SP), the neuritic plaque (NP) is likely to have the greatest significance in the pathology of AD [36]. Normal brain and the early stages in the pathology of AD are largely characterized by diffuse non-neuritic types of plaque [36] and it has also been questioned whether the classic 'cored' type of SP has any significance in AD [34]. Hence, if it is the appearance of NP that is most significant in the pathology of AD, then it is important to establish the relationship between the NP, observed in silver-stained preparations, and the A β deposit subtypes revealed by immunolabelling. Studies of the correlations between the NP found in silver-stained sections with the various types of A β deposit suggest a complex relationship between NP and the primitive A β deposits [37]. There are at least two types of NP, i.e., type A in which A β is in the form of amyloid and the deposit has become associated with dystrophic neurites containing paired helical filaments (PHF) [3] and type B, which contain very little amyloid, and in which the plaque originates from extracellular NFT; the NFT acting as a focus around which dystrophic neurites cluster [38]. This type of NP is also found in normal brain and hence, its significance to the pathology of AD remains unclear. Previous studies suggest that it is the A type of NP which is most closely analogous to the primitive type of A β deposit [3].

The close association between cycles of A β deposition and neuroanatomy has two implications for the pathogenesis of AD. First, the regular cyclic pattern of A β deposition in AD, and especially of the primitive deposits, appears to reflect its development in relation to neurons associated with specific neuroanatomical pathways. This hypothesis is supported by the observations first, that NFT in AD are highly region specific, lamina specific, and cell-type specific [39] and second, that neurons affected by NFT are functionally related suggesting the spread of degeneration across normal synaptic boundaries [40]. In addition, loss of synaptic markers has been observed in AD, especially in laminae III and V of the cerebral cortex which are likely to contain the majority of cells of origin of the long and short cortico-cortical projections [41]. Hence, the cyclic fluctuations in A β deposits appear to have their origin in the degeneration of specific anatomical pathways. Second, diffuse and primitive deposits have a different pattern of cyclic fluctuations compared with the classic deposits which could reflect the association of the classic deposits with the degeneration of blood vessels and the diffuse/primitive deposits with neurons. A possible factor that could influence the results is that large diffuse patches of A β immunolabelling, which could represent a variant of diffuse deposits, were not quantified suggesting that 'diffuse-type' deposits may have a more widespread distribution in the tissue than suggested by the present data. These types of deposit are often large, irregular in shape, without easily defined borders, and are therefore difficult to count. A method of quantification based on measuring area or coverage of these deposits [17] will be necessary to assess their contribution to A β deposition as a whole.

The present data support the hypothesis that A β deposits may form in response to the degeneration of specific anatomical pathways and therefore that neuron connectivity is necessary for their formation. Recently, however, transgenic APP23 embryonic neural cells

were grafted into the neocortex and hippocampus of transgenic mice [42]. Amyloid plaques, mainly of the diffuse type, were formed as early as three months after grafting. These results suggest that diffusion of soluble A β may also be involved in the spread of A β pathology.

In conclusion, spatial analysis of A β deposition parallel to the pia mater or alveus in the temporal lobe by Fourier (spectral) analysis reveals sinusoidal fluctuations in density. Fluctuations occur at different scales, the most common pattern being a small-scale sinusoidal fluctuation repeating every 500–1000 μ m and a larger-scale pattern repeating at distances greater than 1000 μ m. All types of deposit reveal these fluctuations but it is the primitive deposits, which are related to the neuritic SP that have most significance for the pathology of AD. The data support the hypothesis that diffuse and primitive A β deposition is related to anatomical connectivity and most specifically to the degeneration of the callosal and cortico-cortical projections. Hence, Fourier analysis as suggested by Bruce et al [19] may be of value in studying the detailed distribution of A β deposits along or across cortical gyri in AD and in transgenic models of disease and may further help to elucidate the role of A β deposition and the various plaque types in AD.

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List of abbreviations

Aβ	β -amyloid
AD	Alzheimer's disease
APP	Amyloid precursor protein
Apo D	Apolipoprotein D
Apo E	Apolipoprotein E
CA	Cornu Ammonis
ICAM1	Intercellular adhesion molecule 1
CERAD	Consortium to Establish a Registry of Alzheimer's Disease
HSPG	Heparin sulphate proteoglycan
ITG	Inferior temporal gyrus
KS	Kolmogorov-Smirnov statistic
LOT	Lateral occipitotemporal gyrus
MTG	Middle temporal gyrus
NP	Neuritic plaques
NINCDS/ADRDA	National Institute of Neurological and Communicative Disorders and Stroke and Alzheimer's Disease and Related Disorders Association
NFT	Neurofibrillary tangles
PHF	Paired helical filaments
PHG	Parahippocampal gyrus

SP	Senile plaques
STG	Superior temporal gyrus

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Fig 1.
The three major types of β -amyloid ($A\beta$) deposit: a) diffuse ('pre-amyloid'), b) primitive, and c) classic ('dense-cored') deposits ($A\beta$ immunohistochemistry, bar = $1\mu\text{m}$).

Fig 2.
The fluctuating density (number of deposits per $200 \times 1000\mu\text{m}$ plot) of the diffuse beta;- amyloid (A β) deposits parallel to the pia mater along the upper laminae of the MTG in case C.

Fig 3.

The frequency distribution of the spectrogram values derived from the data shown in Fig 2. Goodness of fit of the negative exponential distribution (N = 84, Fisher-Kappa statistic = 8.345, Kolmogorov-Smirnov KS = 0.43; $P < 0.001$ indicating a significant departure from an exponential distribution).

Fig 4.

Spectral density at each period for the data shown in Fig 2. Three significant peaks are evident: 1) at a period of 2.62 units representing a complete A β deposit density cycle every 524µm (i.e., $2.62 \times$ basic field size of 200µm), 2) at a period of 12 units representing fluctuation of deposits on a larger scale with cycles recurring every 2400µm, and 3) maximum spectral density at a period of 42 units representing an even larger scale of fluctuation repeating every 8400µm.

Table 1

Demographic data and cause of death of the cases used in the study.

Case	Gender	Age	Onset	Cause of death	Braak	Apo E
<u>AD</u>						
A	F	91	85	Bronchopneumonia	VI	3/4
B	F	86	83	Bronchopneumonia	VI	3/4
C	F	85	80	Bronchopneumonia	VI	4/4
D	F	88	72	Bronchopneumonia	VI	3/4
E	F	81	77	Bronchopneumonia	V	3/4
F	F	93	91	Bronchopneumonia	VI	4/4
<u>Con</u>						
G	F	90	-	Myocardial infarction	II	3/3
H	M	85	-	Ischaemic heart disease	III	3/4
I	F	85	-	Pulmonary embolism	I	3/3
J	M	84	-	Peritonitis	II	3/3
K	F	80	-	Pulmonary embolism	NA	NA
L	F	83	-	Pulmonary embolism	II	3/3

Abbreviations: Apo E = Apolipoprotein E, Con = Control, M = Male, F = Female, NA = Data not available

Summary of Fourier (spectral) analysis for all brain areas and cases. Data are the frequency of analyses in which a particular pattern was observed.

Table 2

Cases	N	Insufficient data	No significant fluctuations	Corrected for linear trend	Number of significant frequency components		
					One	Two	≥Three
AD	108	9	18	(25)	9	52	20
Con	30	3	12	(3)	1	4	10

Abbreviations: AD = Alzheimer's disease, Con = Control cases, N = number of brain regions analysed

Table 3

Summary statistics for the periods of the first, second, and third and greater frequency components for the Alzheimer's disease (AD) and control (Con) cases (M = Multiple modes present, SD = Standard deviation)

Statistic	Group	Frequency component		
		One	Two	≥Three
Mean (μm)	AD	901	3534	5919
	Con	140	1081	2109
Median (μm)	AD	600	2112	5600
	Con	122	800	2300
Mode (μm)	AD	600	M	M
	Con	100	800	M
Range (μm)	AD	392–7800	600–16800	1600–14000
	Con	100–300	320–3700	450–3700
SD	AD	974	3553	3128
	Con	54	927	1061
Skew	AD	5.05	2.19	0.66
	Con	2.23	2.11	−0.46
Kurtosis	AD	32.05	4.61	0.49
	Con	5.20	5.13	−0.03

Table 4

Average period (µm) of the first two frequency components in different regions of the temporal lobe viz., superior temporal gyrus (STG), middle temporal gyrus (MTG), inferior temporal gyrus (ITG), lateral occipitotemporal gyrus (LOT), parahippocampal gyrus (PHG), and CA sectors of the hippocampus (HC) in the Alzheimer’s disease cases.

Brain region							
Aβ Subtype	Component	STG	MTG	ITG	LOT	PHG	HC
Diffuse	First	424	500	569	754	542	610
	Second	6830	2143	4794	3800	1026	1995
Primitive	First	878	600	935	609	510	721
	Second	3445	4625	5617	2625	2238	2478
Classic	First	578	845	1576	1156	1775	1054
	Second	3408	1660	5125	3100	4800	4400

Analysis of variance (ANOVA): First period, Between brain regions $F = 0.36$ ($P > 0.05$), Between Aβ subtypes $F = 4.13$ ($P < 0.05$), Interaction $F = 0.54$ ($P > 0.05$); Second period, no significant differences.