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Anatomical correlates of the distribution of the pathological changes in the neocortex in Alzheimer disease

(neurofibrillary tangles/lamination/clustering)

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ABSTRACT **The numbers and distribution of the neurofibrillary tangles and neuritic plaques have been determined in several areas of the neocortex in brains affected by various degrees of severity of Alzheimer disease. The homotypical cortex of the "association" areas of the temporal, parietal, and frontal lobes are severely involved, whereas the motor, somatic sensory, and primary visual areas are virtually unaffected. The neurofibrillary tangles are mainly in the supra- and infragranular layers, particularly in layers III and V. In all areas except area 18 in the occipital lobe, there are approximately twice as many tangles in layer V as in layer III. The tangles are arranged in definite clusters, and those in the supra- and infragranular layers are in register. The neuritic plaques occur in all layers but predominantly affect layers II and III and do not show clustering. These data on the severity of the pathological involvement in different areas of the neocortex and the laminar distribution and the clustering of the tangles support the suggestion that the pathological changes in Alzheimer disease affect regions that are interconnected by well-defined groups of connections and that the disease process may extend along the connecting fibers. The invariable and severe involvement of the olfactory areas of the brain in this disease is in striking contrast to the minimal changes in the somatic sensory and primary visual areas and raises the possibility that the olfactory pathway may be initially involved.**

Certain features of the distribution of the pathological changes in the brains of patients with Alzheimer disease suggest that the disease process may affect areas of the cerebral cortex that are interconnected by well-defined sets of corticocortical connections. Together with the hippocampal formation and the cortex and amygdaloid nuclei related to the olfactory system, all of which are in the ventromedial part of the temporal lobe, it is the homotypical cortex in the parietotemporal lobe and in parts of the frontal lobe and the cingulate cortex that are severely affected (1–3). The motor, somatic sensory, and primary visual areas are virtually uninvolved. The neurofibrillary tangles in the cell bodies of neurons and the neuritic plaques affecting the terminal parts of axons are the chief diagnostic features of the disease and both are present in these cortical areas.

If the distribution of the pathological features in the cortex is related to the corticocortical fibers, one would expect a high degree of concordance with certain recent experimental observations on these connections. The regions of the cortex that are affected are clearly the homologues of those that have been shown experimentally in the monkey to be connected by a sequence of links which begins in the main sensory areas and passes through "association" areas of the parietotemporal and frontal lobes to the cingulate cortex and the hippocampal formation (4). Each successive step in this orderly sequence in the parietotemporal lobe is reciprocally connected with the corresponding step in the frontal lobe. In addition, the hippocampal formation and adjoining areas of cortex send fibers back to several of these association areas (5, 6). The least severely affected regions in Alzheimer disease are at the beginning of this sequence, and the most severe at the end, with the association areas occupying an intermediate position and affected to an intermediate degree (2). The tangles that are found in these areas of cortex could be in the cell bodies of the neurons giving rise to these corticocortical fibers, and the plaques could be at the ends of the fibers and their collateral branches. Similarly, in subcortical sites the tangles and plaques in the septum would reflect the reciprocal connections with the hippocampal formation (7, 8). The tangles in the cells of the basal nucleus of the forebrain and of the raphe nuclei and locus ceruleus of the brain stem would be in cells that send fibers to the cortex (9–12). The plaques in the striatum could be indicative of degeneration of corticostriatal fibers (13), and those in the hypothalamus and mamillary bodies, of involvement of the heavy projections to these sites from the amygdala (14) and hippocampal formation (15), respectively. The absence of plaques in the spinal cord (1) would have been predicted because of the minimal involvement of the sensorimotor cortex. If the pathological process is related to these fiber connections of the cortex, it would be reasonable to expect that the intracortical distribution of the plaques and tangles would also be in accord with recent experimental findings on the origins and terminations of these fibers. Thus it can be accepted from the observations made in several experimental studies that there is a differential laminar origin of the various types of efferent fibers from the cortex (16), that both the origins and the terminations of particular sets of corticocortical pathways are in clusters or bands (17) and that there is a differential distribution of callosal and commissural (anterior and hippocampal) fibers between and within areas (18–20).

In an attempt to determine the possible relationship of these anatomical features to the pathological process, quantitative studies have been made of the severity, the lamination, and the degree of clustering of the tangles and, to a lesser extent, of the plaques in different areas of the cortex in brains with various degrees of severity of the disease. In the meantime, the significance of such an anatomical correlation has been shown by Van Hoesen and his colleagues (21), who found the pathological changes in the hippocampus and adjoining areas to affect specifically the cells of origin of the afferent and efferent fibers of this region.

MATERIALS AND METHODS

Blocks were taken from several areas of the neocortex and the hippocampal formation of a number of brains that had been diagnosed as being affected by Alzheimer disease on the

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basis of the clinical assessment and the presence of numerous neurofibrillary tangles and neuritic plaques in sections of the brains. The brains had been fixed by immersion in formalin for 4 weeks and were then sliced in the coronal plane at 1-cm intervals. The blocks were bisected and one half of each was embedded in paraffin wax; 10-μm sections were cut perpendicular to the surface of the cortex and stained by a modification of the Palmgren method, which selectively stains the neurofibrillary tangles (22). From several blocks a 1:10 series of sections extending over a few millimeters was mounted and stained. From the other half of each block 25-μm sections were cut and stained by the Von Braunmuhl technique (34) to show neuritic plaques.

The areas of neocortex from which blocks and sections were taken in most brains included Brodmann's areas 9, 46, 6, and 4 and the posterior orbital cortex of the frontal lobe; the primary somatic sensory cortex and areas 39 and 7 of the parietal lobe; areas 20, 21, and 38 of the temporal lobe; and areas 17 and 18 of the occipital lobe (35).

Quantitative studies on the number and distribution of the tangles and plaques were done in two ways. In the first method, the position of each tangle or plaque was marked on a projected outline of the section of 4–6 mm width, using a Leitz Micropromar projector. For series of paraffin sections that had been stained, this procedure was done on each section and features such as blood vessels and the fundus of a sulcus were used as reference points. The numbers of tangles and plaques in the supra- and infragranular layers in 100-μm widths of the sections were counted by placing a transparent grid over the projected outline. In the second method, a graticule was placed in the eyepiece and the tangles and plaques were counted, at magnifications of $\times$200 and $\times$80, respectively, in 10 randomly selected fields of each section. Most of the quantitative studies have been made on the tangles rather than the plaques because of the evidence that they are more closely correlated with the severity of the clinical condition and with the cholinergic deficit than are the plaques (23).

RESULTS

Qualitative examination of sections from all the neocortical areas that have been studied makes it clear that the majority of the tangles are in the supra- and infragranular layers, and particularly in layers III and V, with layer IV virtually clear between them. In most areas, layer V has more than layer III, but in area 18 of the occipital lobe the reverse is found. As far as can be judged with light microscopy, the majority, if not all, of the tangles are in pyramidal cells. The quantitative data confirm these qualitative impressions. Table 1 shows the data and analysis for the cortex of the middle temporal gyrus, area 21; comparable data for other areas of temporal, parietal and frontal cortex were essentially similar. In the less severely affected brains, there are few tangles in either the supra- or infragranular layers, but there are more in the infragranular layers and the difference between the layers may reach a ratio of 2:1. In the more severely affected brains, there is an increase in the number of tangles in both the supra- and infragranular layers, but the difference between them becomes less. Tests of correlation between the numbers of tangles in the supra- and infragranular layers were all significant in rejecting the null hypothesis of no correlation against the one-tailed alternative of positive correlation. For three of the six brains, the tests were significant at the 5% level; for the other three brains, the tests were more significant (2%, 1%, and 0.1% levels, respectively). The differences in number of tangles between the supra- and infragranular layers were tested by *t* tests, and all were very highly significant (0.1% level). In our material, the plaques are present throughout the depth of the cortex, but in most brains and areas they were more numerous in layers II and III.

Both the qualitative and the quantitative studies show that in sections of all the areas of neocortex that were examined, the tangles are arranged in definite clusters and that the clusters in the supra- and infragranular layers are in register (Table 1). Analysis of the data confirms that the tangles are arranged in a nonrandom manner. The randomness of the distribution of the tangles was examined first by dispersion tests. In all six brains, there was evidence of clustering in both the supra- and infragranular layers. For 10 out of the 12 layers, significance reached the 0.1% level, and for the remaining two, the 1% and 5% levels. The shape of the distributions of numbers of tangles is suggestive of a mixture of two random processes operating with two densities; this was confirmed by fitting a mixture of two Poisson distributions and testing the goodness of fit with χ^2 tests to the supra-

Table 1. Data* and analysis of tangles in area 21

Case					Tangles per strip				Goodness of fit for two-term Poisson model				
No.	Sex	Age	Layers†	Sample‡ (n)	Mean ($\bar{x}$)	Variance (s^2)	D§	P	χ^2	d.f.¶	P	r‖	P
1	M	51	Supra	1090	1.36	1.74	128	<0.001	7.5	4	NS	0.0636	<0.02
			Infra	1090	2.42	2.64	109	<0.01	17.8	6	0.01		
2	F	67	Supra	215	3.13	4.60	147	<0.001	12.4	6	0.01	0.1801	<0.01
			Infra	215	4.09	6.23	152	<0.001	5.74	7	NS		
3	F	80	Supra	716	0.23	0.27	117	<0.01	0.2	1	NS	0.0602	≈0.05
			Infra	716	0.47	0.51	108	≈0.05	2.8	1	NS		
4	M	89	Supra	578	0.80	1.03	129	<0.001	0.2	2	NS	0.0744	<0.05
			Infra	578	2.12	2.67	126	<0.001	2.6	5	NS		
5	F	93	Supra	2380	0.27	0.30	111	<0.001	0.5	1	NS	0.0379	<0.05
			Infra	2380	0.39	0.43	110	<0.001	4.7	2	NS		
6	F	95	Supra	1962	0.37	0.44	119	<0.001	2.5	1	NS	0.0757	<0.001
			Infra	1962	0.82	0.91	111	<0.001	1.1	2	NS		

NS, not significant.
*Obtained by the projection-outline method (see *Materials and Methods*).
†Supra and Infra refer to the supragranular and infragranular layers of the cortex.
‡No. of 100-μm strips.
§Dispersion index = $(s^2/\bar{x}) \times 100$.
¶Degrees of freedom.
‖Correlation coefficient between supra- and infragranular layers.

Table 2. Means and standard deviations of tangle counts in visual cortex

	Tangles per field (n = 10)			
	Area 18 (peristriate)		Area 17 (striate)	
Case*	Supra	Infra	Supra	Infra
1	0.8 ± 2.1	0	0	0
2	0.8 ± 1	1.7 ± 1.2	0.1 ± 0.3	0
3	0.3 ± 0.9	0.2 ± 0.4	0	0
4	5.8 ± 3.1	4.1 ± 2.5	0.1 ± 0.3	0
5	11.3 ± 10.5	4.2 ± 2.4	0.5 ± 0.7	0.4 ± 0.9
6	11.2 ± 7.9	3.0 ± 2.3	5.3 ± 1.8	0.6 ± 0.8
7	12.4 ± 4.4	6.8 ± 2.7	1.8 ± 1.6	0.5 ± 0.7
8	24.6 ± 7.4	14.5 ± 5.6	6.7 ± 4.9	2.7 ± 2.5

These data were obtained by the graticule method (see *Materials and Methods*). Supra and Infra refer to the supragranular and infragranular layers of the cortex.

*It should be noted that these are *not* the same brains as those in Table 1.

and infragranular layers of the six brains. Only in the cases of one infragranular layer, significant at the 1% level, and one supragranular layer, significant at the 5% level, was there any evidence of a departure from the two-term Poisson model; for the other 10 layers, this model fit the data well. The plaques appear to be distributed randomly and statistical analysis of the data of one area confirms this ($\chi^2 = 1.29$, 3 d.f., $P > 0.1$).

In any one brain the severity of the pathological change in the neocortex, as judged by the numbers of plaques and tangles, was always greatest in the homotypical cortex of the association areas of the temporal, parietal, and frontal lobes and was least in the sensorimotor and primary visual areas. Quantitative data for tangles in areas 17 and 18 are given in Table 2, from which it can be seen that tangles are only present in area 17, the primary visual area, when the severity in area 18 rises above a certain level. There were too few tangles in the somatic sensory and motor areas for valid quantitative studies. Apart from the neocortex, the entorhinal area, the hippocampus, and the uncus were always severely affected (24) but quantitative data were not taken.

It is difficult to be certain whether callosal and commisural connections are involved, but the usually symmetrical distributions of pathological changes in the cortex and the involvement of the anterior olfactory nucleus and of most of the anteroposterior extent of the hippocampus strongly suggest that they are.

DISCUSSION

The quantitative data and the statistical analysis support the suggestion that the distribution of the pathological changes in the neocortex in Alzheimer disease is related to the cortical and subcortical connections of the affected areas. The observations also extend to the neocortex the conclusion, derived from the study of the hippocampal formation in this disease, that there is a close correlation between the distribution of the degeneration and the anatomical connections (21). They are also in agreement with the finding of a strong correlation between the number of neuritic plaques in certain areas of the neocortex and the degree of cellular degeneration in the related parts of the basal nucleus because these data also indicate that the distribution of the pathological changes in Alzheimer disease has an anatomical basis (25).

Most of the tangles appear to be in pyramidal neurons, and this by itself is significant because these are the cells of origin of most, if not all, of the efferent fibers of the neocortex. Most of the efferent fibers that arise in layers II and III pass to other cortical areas in the same or opposite hemisphere (16), and those on the same side are mainly those that are sometimes called "feed-forward" (26); the tangles in these layers almost certainly represent involvement of some of these connections. From layer V the majority of the fibers pass to subcortical structures such as the striatum, amygdala, and brain stem (16), but a small number of what have become known as reciprocal or "feed-back" corticocortical fibers also arise from layer V and pass to other areas of cortex in the same hemisphere (26). The tangles in this layer indicate involvement of some of these connections and would be in agreement with the presence of plaques in the relevant subcortical sites. The reversal in the numbers of tangles in the supra- and infragranular layers in area 18 of the occipital lobe, with more in the supragranular than in the intragranular, as compared with the areas of association cortex may be due to there being fewer fibers to the amygdala (4) and to there being more feed-back connections between the various visual areas (26). The virtual absence of tangles in layer IV would also be consistent with the suggested relationship of the pathology to the fiber pathways outlined above, because few long efferent fibers arise in this layer (16).

The arrangement of the tangles in clusters, and particularly the fact that those in the supra- and infragranular layers are in register, also support the possible involvement of specific fiber pathways. There is now considerable experimental evidence for the origin of the efferent fibers from the cortex to other cortical and subcortical sites to be in clusters, as seen on a section, and in bands across the cortex, in reconstructions of series of sections (16). Although the tangles were not found to be aligned in bands across the entire extents of the reconstructions of the serial sections, they did form patches over the short distance covered by three or four sections. One reason for not seeing a clear pattern of bands may have been the technical difficulty of aligning a series of sections accurately enough to demonstrate this. The neuritic plaques affect the terminal parts of axons (3), so their distribution through most layers of the cortex and in a random manner is not surprising. The plaques could involve the collateral branches of the efferent fibers as well as afferent fibers from other areas of the cortex and from subcortical structures.

The severity of the pathological changes is always maximal in the hippocampal formation and in the adjoining cortex of the entorhinal area and uncus, and it is least in the sensorimotor and primary visual areas (1–3). If the disease process involves the long fiber connections, it could be in either an orthograde or a retrograde direction or a combination of both in different sets of fibers. If in an orthograde direction, it could be along those fiber pathways that have been described passing from the entorhinal and adjacent areas of cortex to precisely those parts of the parietotemporal, frontal, and cingulate regions that are severely involved (5); it is significant that these fibers arise in layer IV of the entorhinal area where, in addition to tangles, there is severe degeneration and loss of cells (21). If in a retrograde direction, the disease process could involve the sequence of connections that has already been described passing from the sensory areas through the association cortex to the entorhinal area and hippocampal formation (4, 6). Progression in either of these directions along the relevant pathways could certainly account for the relative severity of involvement in the different areas. It would be of interest to determine, by examination of sagittal sections of the supratemporal plane, whether the primary auditory cortex is similar to the somatic and visual areas in being virtually unaffected.

The distribution of the pathology in the cortex and amygdala in the somewhat similar condition of Pick disease is virtually complementary to that in Alzheimer disease (2, 3, 27, 28). The disease process may again be related to certain corticocortical connections, because the regions that are usually the most severely affected in Pick disease, near the

frontal and temporal poles, are reciprocally connected by such connections (4). The frontal and temporal poles also send fibers to the cortex on the orbital surface of the frontal lobe, in the anterior parts of the cingulate gyrus and close to the entorhinal cortex (4), areas which are frequently involved in Pick disease (3, 27). These areas of cortex are also those that receive the heaviest projections from the basolateral group of amygdaloid nuclei (29). In these areas of cortex, the cells in layer III (the origin of corticocortical fibers) are the first and most severely affected in Pick disease, and their appearance is similar to that in retrograde degeneration (3). In both these diseases, therefore, the distribution of the pathology in the neocortex could have a basis in known sets of connections, both cortical and subcortical. In Alzheimer disease, the fibers passing from the cortex on the lateral surfaces of the parietotemporal and frontal lobes, and from the more posterior parts of the cingulate gyrus, to the hippocampal formation (4, 6) would be predominantly affected, together with the olfactory areas of cortex and the medial amygdaloid nuclei. In Pick disease, the connections from the cortex of the frontal and temporal poles and of the orbital surface of the frontal lobe to the hippocampal formation would be mainly involved, as well as the connections of these neocortical areas with each other and with the anterior parts of the cingulate gyrus. Here the basolateral group of amygdaloid nuclei is involved (28), those which are not directly related to the olfactory pathway but which have reciprocal connections with the affected areas of neocortex (29, 30).

The invariable finding of severe and even maximal involvement of the olfactory regions in Alzheimer disease (31) is in striking contrast to the minimal pathology in the visual and sensorimotor areas of the neocortex and cannot be without significance. In the olfactory system, the sites that are affected—the anterior olfactory nucleus (32), the uncus, and the medial group of amygdaloid nuclei—all receive fibers directly from the olfactory bulb (33). These observations at least raise the possibility that the olfactory pathway is the site of initial involvement in the disease. If this is indeed so, the disease process could then spread to involve the hippocampal formation and the association areas of the neocortex in the parietotemporal and frontal lobes through the corticocortical pathways that have been described. If spread also occurred along the subcortical connections of these olfactory areas, the hypothalamus would be predominantly affected and this might lead to disturbance of its endocrine and autonomic functions. The close correlation between the distribution of the pathological changes and the anatomical connections in Pick and Alzheimer diseases suggests that the same may be true in other conditions.

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