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Alzheimer's disease: a correlative study

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SUMMARY In a study of 17 patients with histologically proven Alzheimer's disease the relationship between psychological, pathological and chemical measures of disorder was examined. Severity of dementia, determined by mental test performance, correlated highly with pathological change in large cortical neurons (cell loss and reduction in nuclear and nucleolar volume and cytoplasmic RNA content), to a lesser extent with cortical senile plaque and neurofibrillary tangle frequency and reduction in acetylcholine (ACh) synthesis, and not with reduction in choline acetyltransferase (CAT) activity. A strongly significant relationship was demonstrated between cell loss and reductions in nuclear and nucleolar volume and cytoplasmic RNA content. Reduction in CAT activity and senile plaque frequency were significantly correlated, thereby linking changes in the sub-cortical projection system of the nucleus basalis with the cortical pathology. The pattern of correlations suggests that the dementia of Alzheimer's disease is largely a reflection of the state of large cortical neurons, and it is argued that abnormalities in the latter may not be directly related to primary loss of cholinergic neurons in the subcortex.

Interest in pre-senile dementia has been re-awakened by the finding of several independent groups that in the cerebral cortex of patients with Alzheimer's disease there is a reduction in the activity of the enzyme choline acetyl-transferase (CAT) which catalyses the synthesis of acetylcholine (ACh).[1–4] Post-synaptic muscarinic receptors are apparently not greatly affected,[5] thus raising the possibility of therapy by the use of agents enhancing the activity of the cholinergic system. The implication of a cholinergic specific projection system to the cerebral cortex in the pathogenesis of Alzheimer's disease[6] reinforces such a possibility. However, it cannot necessarily be inferred that deficiencies in cortical CAT activity reflect a reduced ability of the brain to synthesize ACh, and indeed the results of animal studies suggest that CAT may not be the rate limiting enzyme of ACh synthesis.[7] Nevertheless, the concept that the cortical abnormalities of Alzheimer's disease might be secondary to a primary failure of subcortical projection systems to the cortex[8] is attractive because of its theoretical and possibly therapeutic implications.

If it were the case that a cholinergic specific subcortical system is the sole or contributory cause of the psychological changes of Alzheimer's disease then consistent relationships ought to exist between measures of cholinergic activity, the nature and degree of pathological change in the cerebral cortex, and the severity of the dementia. In necropsy series of Alzheimer's disease in elderly subjects the relationship between CAT activity, the frequency of senile plaques and neurofibrillary tangles, and the degree of dementia have been studied,[9–12] but the results have not been in uniform agreement.

The lack of consistency in reported correlations between the neurochemical, pathological and psychological measures in Alzheimer's disease may be partly attributable to methodological differences, the tendency for necropsy studies to include the very old and more severely advanced forms of the disease and for psychological measures to be made some time before pathological and biochemical examination of the brain. Moreover, the effects of terminal illness and postmortem change on the brain may introduce errors into neurochemical assays.

In the present study of patients with Alzheimer's disease in the presenium, psychological assessment provided measures of relative severity of dementia,

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with which chemical and pathological indices of impairment could be compared. Cortical tissue from the right middle temporal gyrus permitted the neurochemical assay of CAT activity, and also the determination of ACh synthesis in cortical synaptosomes. In addition to the pathological measurement of neurofibrillary tangle and senile plaque frequency in the cortex the number of pyramidal nerve cells was counted and the state of their perikarya assessed by estimates of reductions in their nuclear and nucleolar volume and cytoplasmic RNA content.

The assessment of ACh synthesis enabled a more complete description of cholinergic function in the cerebral cortex and its interrelationships with pathological change in large cortical neurons and dendrites, and the degree of dementia.

Methods

Subjects

The study group consisted of 17 patients, 11 female and 6 male, who had presented in the presenium with a progressive deterioration of mental function, and in whom a histological diagnosis of Alzheimer's disease was made following right temporal lobe biopsy.[13] A further patient, with histologically confirmed Alzheimer's disease[13] was not included in the present series since the site of biopsy was the right frontal lobe. The procedure of cerebral biopsy was approved by the Manchester Central District Ethical Committee. At the time of investigation the patients' mean age was 59 years, range 52–69 and mean duration of illness 3·7 years, range 1–8 years.

In all patients impaired memory was a prominent feature. No patient could give the correct date, nor retain a name and address over a one minute interval. In two male patients amnesia was the sole deficit, whereas in others disturbance of perceptuo-spatial and constructional abilities was evident. These patients had difficulty dressing and had become lost in familiar surroundings. Examination revealed difficulty in drawing, in copying hand configurations and calculation. Location and spatial orientation of objects and body parts was generally more severely affected than was perceptual discrimination and identification of objects.

In 10 patients amnesia and visuo-spatial disorder were combined with language disturbance. Speech characteristically was fluent, although output was reduced. Paraphasic errors were noted. Impairment of comprehension, repetition and naming, and also of reading, writing and communication by gesture and pantomime were also evident.

In contrast to profound cognitive difficulties, social conduct was well preserved. There was generally some retention of insight, which appeared inversely proportional to the degree of amnesia. Anxiety and querulousness were common but psychotic symptoms were absent at referral.

Neurological signs of mild degree were observed in a proportion of patients and consisted of akinesia and rigidity, defective tactile localisation and postural awareness.

Electro-encephalograms were abnormal in all patients, with excess slow wave activity, maximal fronto-anteriorly with the emphasis over the temporal lobes.

Computed tomography of the brain revealed the presence of cerebral atrophy without other structural change.

Histological and biochemical analysis

Cerebral cortical biopsy and all subsequent procedures were as previously described,[13] except that other sections were cut at 16 μm thickness and stained with Azure B for measurements of cytoplasmic RNA content, nuclear and nucleolar volume in pyramidal cells.[14] In these sections the number of nucleolated pyramidal nerve cells was also counted in cortical layers III and V in 10 consecutive non-overlapping microscope fields using a Weibel graticule at a magnification of $\times 250$ (equivalent to a field size of 0·176 mm^2) from which the mean number of such cells per field was calculated. These values of packing density were corrected for atrophy by measuring the decrease in cortical thickness, and expressing values as number per unit volume (Nv) according to:—

$$Nv = \frac{n}{a} \times \frac{1000}{t} \times \frac{D_1}{D_0}$$

where n = mean number of nucleolated nerve cells per field
a = area of measuring field (0·176 mm^2)
t = nominal section thickness (16 μm)
D_0 = mean cortical thickness in control patients (3·43 mm)
D_1 = mean cortical thickness in Alzheimer's disease patients. This ranged from 1·45 to 3·24 mm, mean 2·33 $\pm$ 0·47 mm, representing a 32·3% ($p < 0·001$) decrease compared to control values.

Percentage loss of pyramidal cells from both layers III and V and percentage decreases in nuclear and nucleolar volume and cytoplasmic RNA content were calculated for each of the Alzheimer's disease patients by comparing individual patient values with the corresponding mean value calculated from five control patients.

Table 1 *Frequency of senile plaques and neurofibrillary tangles in temporal cortex of patients with Alzheimer's disease*

Patient	*SP (n/mm^2)*	*NT (n/mm^2)*
A	2·6	3·1
B	6·7	12·9
C	17·3	25·2
D	19·8	31·3
E	12·3	26·8
F	17·7	26·7
G	21·1	30·0
H	18·7	29·4
I	10·8	0·2
J	42·7	54·2
K	34·4	19·6
L	16·4	14·7
M	19·2	35·7
N	9·3	23·6
O	8·4	15·7
P	13·4	28·8
Q	51·4	38·4
Control mean	0	0

Psychological testing

A rating of magnitude of dementia from 0–9 was obtained on the basis of patients' mental test performance as previously described.[13] Language, perceptuo-spatial functions and memory were each rated from 0–3 in terms of extent of impairment, and the overall clinical rating represented a cumulation of these three assessments. Additional performance measures were obtained from the Wechsler Adult Intelligence Scale (WAIS),[15] a modified version of the Token Test,[16] and a continuous Visual Reaction Time task, involving key press responses to computer generated random digits on a video monitor. These latter tests were chosen owing to their demands respectively on linguistic and perceptuo-spatial functions, known to be impaired in the patient group. Moreover, they provide an easily quantifiable measure of performance.

Actual scores on the WAIS clearly may not provide an accurate reflection of extent of acquired disorder since these take no account of premorbid level of performance. Reading vocabulary, frequently used to estimate premorbid intellectual function[17] was however impaired in several patients in whom an acquired dyslexia was combined with visuo-spatial deficits, and therefore was unhelpful in determining the extent of decline. Nevertheless, the occupational and educational status of patients in the study group was sufficiently homogeneous, and the spectrum of performance on the WAIS sufficiently broad, that ranking of patients according to scores appeared to provide an appropriate measure of relative severity.

Statistics

Correlative analyses were carried out using Spearman's nonparametric correlation statistic (r), based on ranks.[18] A one-tailed test of significance was applied, in accordance with the unidirectional prediction that an increase in clinical severity of dementia corresponds to an increase in pathological and chemical measures of disorder. Comparisons between data from Alzheimer's disease and control groups were carried out using a two-tailed Student's *t* test.

Results

Neuropathological

Cortical sections from the 17 patients were found on light microscopy to contain the characteristic changes of Alzheimer's disease, namely senile plaques and neurons bearing neurofibrillary tangles. Senile plaques were most frequently observed in layers I–III in the cerebral cortex and neurofibrillary tangles in neurons of all cortical layers (table 1), but were not present in the five biopsy controls. In Alzheimer's disease patients senile plaque and neurofibrillary tangle frequencies were significantly correlated ($r = 0{\cdot}78$, $p < 0{\cdot}001$).

The number of pyramidal cells in cortical layers III and V and measures of nuclear volume, nucleolar volume and RNA content in such cells, hereafter termed "perikaryal" measures, are shown in table 2. The number of pyramidal nerve cells was reduced compared to control values by an average of 60% in layer III and 56% in layer V. Nuclear volume was reduced in the pyramidal cells of layers III and V by 38% and 36% respectively, nucleolar volume by 27% and 34% and RNA content by 22% and 25%. Correlations were performed between all possible combinations of percentage decrease in cortical thickness, pyramidal cell frequency and their nuclear and nucleolar volume and cytoplasmic RNA content in

Table 2 *Pyramidal cell counts and measures of perikaryal function in temporal cortex of patients with Alzheimer's disease*

Patient	*Frequency of pyramidal cells**		*Nuclear volume†*		*Nucleolar volume†*		*Cytoplasmic RNA†*	
	layer III	*layer V*	*layer III*	*layer V*	*layer III*	*layer V*	*layer III*	*layer V*
A	10·3	16·5	1512 ± 54	1611 ± 41	17·1 ± 0·5	17·6 ± 0·4	34·1 ± 1·0	36·2 ± 0·9
B	10·0	13·3	1490 ± 67	2015 ± 64	15·7 ± 0·5	14·7 ± 0·5	33·3 ± 0·9	34·0 ± 0·9
C	8·9	15·9	1304 ± 47	1439 ± 53	14·1 ± 0·6	14·5 ± 0·4	31·0 ± 0·8	32·6 ± 0·9
D	5·9	9·4	1115 ± 31	1325 ± 40	11·1 ± 0·3	10·8 ± 0·3	26·4 ± 0·9	27·5 ± 0·9
E	7·2	9·6	1068 ± 26	1146 ± 46	12·0 ± 0·4	11·5 ± 0·5	27·3 ± 0·8	27·6 ± 0·8
F	8·4	12·0	1612 ± 80	1838 ± 58	14·7 ± 1·0	12·9 ± 0·5	32·7 ± 1·0	29·7 ± 0·9
G	11·3	13·7	816 ± 23	1531 ± 38	12·9 ± 0·4	13·3 ± 0·4	28·0 ± 0·9	27·0 ± 0·9
H	7·4	10·9	837 ± 35	1099 ± 31	16·0 ± 0·8	12·8 ± 0·4	28·9 ± 0·7	28·7 ± 0·7
I	9·5	11·8	1119 ± 39	1202 ± 35	12·9 ± 0·6	12·4 ± 0·6	26·8 ± 0·8	27·7 ± 0·9
J	2·4	3·9	1165 ± 54	1059 ± 40	13·1 ± 0·7	9·2 ± 0·3	27·1 ± 0·7	24·1 ± 0·7
K	8·0	10·9	942 ± 25	942 ± 39	11·2 ± 0·4	11·0 ± 0·3	28·5 ± 0·9	27·6 ± 0·9
L	4·4	8·2	747 ± 23	1150 ± 31	10·3 ± 0·4	9·8 ± 0·3	28·0 ± 0·9	31·1 ± 0·7
M	5·3	8·6	599 ± 31	675 ± 39	13·9 ± 0·9	10·9 ± 0·5	26·7 ± 0·7	28·1 ± 0·8
N	4·5	6·9	913 ± 47	1122 ± 52	13·1 ± 0·6	10·2 ± 0·4	27·2 ± 0·8	25·1 ± 0·8
O	5·3	6·8	1071 ± 65	1431 ± 90	12·8 ± 0·7	11·4 ± 0·5	26·6 ± 0·7	27·5 ± 0·8
P	5·1	8·5	1009 ± 22	1111 ± 25	9·8 ± 0·2	10·7 ± 0·3	28·3 ± 0·9	26·7 ± 0·9
Q	3·6	5·6	821 ± 23	1182 ± 26	10·9 ± 0·3	9·9 ± 0·2	23·9 ± 0·8	24·7 ± 0·9
Control mean	17·4 ± 0·6	23·2 ± 0·2	1714 ± 71	2018 ± 136	17·7 ± 0·5	18·1 ± 0·3	36·3 ± 0·7	37·9 ± 0·6

* units are $n \times 10^{-3}$ per mm^3
† units are μm^3
± standard error

Table 3 *Correlations between reductions in cortical thickness, pyramidal cell number and perikaryal change in Alzheimer's disease*

	Nuclear volume III	*Nuclear volume V*	*Nucleolar volume III*	*Nucleolar volume V*	*RNA content III*	*RNA content V*	*Cortical thickness*	*Pyramidal cells III*	*Pyramidal cells V*
Nuclear volume III	—								
Nuclear volume V	0·59**	—							
Nucleolar volume III	0·46*	0·31	—						
Nucleolar volume V	0·50*	0·66**	0·67**	—					
RNA content III	0·44*	0·36	0·56**	0·64**	—				
RNA content V	0·35	0·44*	0·56**	0·69***	0·69***	—			
Cortical thickness	0·28	0·42*	0·30	0·74***	0·50*	0·48*	—		
Pyramidal cells III	0·41*	0·61**	0·52*	0·94***	0·57**	0·60**	0·79***	—	
Pyramidal cells V	0·43	0·57**	0·56**	0·92***	0·70***	0·72***	0·80***	0·95***	—

Spearman's (r) rank correlation.
*** = $p < 0·001$; ** = $p < 0·01$; * = $p < 0·05$.

both layers III and V (table 3). Percentage reduction in nucleolar volume was highly correlated with reduction in cytoplasmic RNA in both layers, perhaps not an unexpected finding since the ribosomal RNA of the cytoplasm is produced within the nucleolus. In contrast, correlations between changes in nuclear volume and both nucleolar volume and cytoplasmic RNA content were generally weak or non-significant. Reduction in cortical thickness related most strongly to the extent of nerve cell loss from both layers III and V of the cortex, and more weakly to perikaryal change in surviving cells, while a strong relationship was demonstrated between reductions in frequency of pyramidal cells and in perikaryal indices.

Senile plaque frequency was significantly correlated with perikaryal measures in layer V: an increase in plaques corresponded to an increase in the degree to which perikaryal measures were reduced ($r = 0·43$, $p < 0·05$; $r = 0·43$, $p < 0·05$; $r = 0·48$, $p < 0·05$ for nuclear volume, nucleolar volume and cytoplasmic RNA content respectively). Plaque frequency did not correlate significantly with perikaryal measures in layer III, nor with cell loss in layers III and V. Neurofibrillary tangle frequency correlated significantly with cell loss in layers III and V and with perikaryal measures in layer V: an increase in tangles corresponding to an increase in extent of cell loss ($r = 0·47$, $p < 0·05$; $r = 0·43$, $p < 0·05$ for layer III and layer V respectively), and reduction of perikaryal measures ($r = 0·42$, $p < 0·05$; $r = 0·45$, $p < 0·05$; $r = 0·60$, $p < 0·01$) for nuclear and nucleolar volume and RNA content in layer V respectively. A significant relationship between tangle frequency and RNA content in layer III was also demonstrated ($r = 0·46$, $p < 0·05$).

Biochemical

ACh synthesis and CAT activity were reduced in Alzheimer's disease patients (table 4), the mean values for synthesis and enzyme activity being respectively 41% and 39% of control values, statistically significant at $p < 0·001$ (Student's t test). Values of ACh synthesis and CAT activity were significantly correlated ($r = 0·55$, $p < 0·05$).

Pathological-biochemical correlates

Reduction in CAT activity correlated significantly with increased senile plaque frequency and to a lesser degree neurofibrillary tangle frequency (table 5). Reduction in ACh synthesis was not related to plaque or tangle frequency. Although reductions in both CAT activity and ACh synthesis correlated with loss of pyramidal cells there was not a consistent relationship between biochemical and "perikaryal" measures of disorder. Significant correlations were found only between measures of ACh synthesis and percentage reductions in nuclear and nucleolar volume in layer V.

Table 4 *CAT activity and ACh synthesis in temporal cortex of patients with Alzheimer's disease*

Patient	*CAT activity (nmol/min/mg)*	*ACh synthesis (dpm/min/mg)*
A	4·20	4·60
B	3·80	3·13
C	2·70	2·39
D	1·08	3·96
E	4·45	4·18
F	na	2·46
G	3·92	4·35
H	na	3·30
I	4·84	2·61
J	na	2·19
K	3·23	2·39
L	2·40	1·84
M	na	1·27
N	na	2·20
O	na	1·77
P	3·99	2·92
Q	0·93	2·21
Control mean + SD	8·2 + 2·4 (7)	6·9 + 1·3 (13)
range	5·4 – 12·6	5·2 – 8·7

na = data not available

Table 5 *Pathological-chemical correlations*

	Senile plaques	*Neurofibrillary tangles*	*Pyramidal cell loss*		*Nuclear volume*		*Nucleolar volume*		*Cytoplasmic RNA*	
			layer III	*layer V*	*layer III*	*layer V*	*layer III*	*layer V*	*layer III*	*layer V*
CAT activity	−0·69*	−0·55*	−0·55*	−0·46	−0·37	−0·03	−0·42	−0·49	−0·24	−0·26
ACh synthesis	−0·19	−0·10	−0·64†	−0·62†	−0·35	−0·42*	−0·22	−0·61†	−0·41	−0·22

Spearman rank correlation coefficients: r
† = $p < 0{\cdot}01$; * = $p < 0{\cdot}05$.
Correlations with CAT activity are based on values from 11 patients, and with ACh synthesis from 17 patients.
A high level of disorder is represented for chemical measures by low values and for pathological measures by high values, hence the inverse correlations.

Neuropsychological

Patients differed in terms of severity of dementia, clinical ratings ranging from a score of 2 in the two male patients with a selective amnesic disorder to 9 in a female patient with a combination of profound memory, perceptuo-spatial and language disturbance (table 6). Verbal and Performance scores on the WAIS, Token Test and Reaction Time measures also demonstrated different degrees of impairment in the patient group. The discriminatory power of these tests was limited by the floor level performance observed in the three most severely affected patients. Nevertheless, the tests were able successfully to distinguish between mild, moderate and severe degrees of disability, and inter-test correlations and correlations between test measures and clinical ratings of severity of dementia were significant at least at the $p < 0{\cdot}01$ level, with the exception that Verbal and Performance scales of the WAIS yielded a correlation of only 0·57, $p < 0{\cdot}05$. The strongest relationship was demonstrated between WAIS Performance scale scores and reaction time measures, $r = -0{\cdot}92$, $p < 0{\cdot}001$, prolonged response latencies associated with low Performance scores.

Psychological-neuropathological correlates

A strong and consistent relationship was demonstrated between psychological and "perikaryal" measures: poor psychological performance was associated with loss of pyramidal cells and reduced nuclear and nucleolar volume and RNA content (table 7). Weaker and less consistent relationships were demonstrated between psychological measures and measures of senile plaque and neurofibrillary tangle frequency. Pathological correlations with the severity of dementia, as measured by the clinical rating, are depicted in fig 1.

Psychological-biochemical correlates

Measures of ACh synthesis correlated significantly with clinical rating of dementia severity, and the visual reaction time test performance: low values of ACh synthesis corresponding to a high rating of dementia severity and prolonged response latencies (table 8).

Table 6 *Psychological performance in patients with Alzheimer's disease*

Patient	*Clinical rating of severity*	*WAIS*		*Token test % correct*	*Continuous reaction time*
		Verbal	*Performance*		
A	2	123	99	96	2·0
B	2	97	95	99	2·3
C	4	96	73	65	5·6
D	4	77	53	16	40·7
E	5	65	nt	7	48·0
F	5	83	53	63	4·5
G	6	68	63	18	3·0
H	6	nt	nt	nt	nt
I	6	65	50	28	17·7
J	6	56	59	23	9·5
K	7	70	52	40	18·8
L	7	58	54	40	28·5
M	7	81	nt	nt	nt
N	7				49·0
O	7	81	nt	15	nt
P	8	nt	nt	nt	nt
Q	9	nt	nt	nt	nt

nt = not testable. For statistical purposes this was regarded as synonymous with "floor" level performance, and patients were ranked most severely affected.
A normal "non-demented" score on the Clinical rating scale is 0. For the Visual reaction time task mean RT obtained from 5 non-demented control subjects (mean age 52 years) was 1·5 s (range 1·2–1·8 s).

Table 7 *Psychological-pathological correlations*

	Senile plaques	*Neurofibrillary tangles*	*Pyramidal cell loss*		*Nuclear volume*		*Nucleolar volume*		*Cytoplasmic RNA*	
			layer III	*layer V*	*layer III*	*layer V*	*layer III*	*layer V*	*layer III*	*layer V*
Clinical rating (17)	0·33	0·27	0·67†	0·71‡	0·71‡	0·61†	0·62†	0·71‡	0·50*	0·60†
WAIS verbal (16)	−0·45*	−0·47*	−0·61†	−0·63†	−0·56*	−0·62†	−0·56*	−0·68†	−0·43*	−0·65†
WAIS performance (16)	−0·16	−0·33	−0·53*	−0·59†	−0·52*	−0·60†	−0·42	−0·47*	−0·56*	−0·47*
Token test (16)	−0·37	−0·65†	−0·58†	−0·61†	−0·66†	−0·62†	−0·43*	−0·54*	−0·65†	−0·64†
Visual RT test (17)	0·18	0·39	0·67†	0·70‡	0·61†	0·64†	0·45*	0·59†	0·60†	0·47*

‡ = $p < 0·001$; † = $p < 0·01$; * = $p < 0·05$.
( ) = number of patients on which correlations based.
High levels of impairment are represented for pathological measures and for the clinical rating and reaction time test by high values; for other psychological measures high impairment is represented by low scores, hence the inverse correlations.

No significant correlation was demonstrated between ACh synthesis and other psychological measures. Measures of CAT activity did not correlate significantly with any of the psychological measures.

The relationship between ACh synthesis and clinical rating of dementia is illustrated in fig 1.

Relationship between duration of symptoms and psychological, pathological and chemical measures of disease severity

There were no significant correlations between the length of illness and severity of disorder, as measured by psychological, pathological and chemical indices.

Discussion

The relationship between psychological, pathological and chemical indices of disease has been examined in a group of 17 patients with Alzheimer's disease who shared common pathological and biochemical changes in the cerebral cortex. In accordance with the prediction that increased severity in terms of one index would correspond to increased severity in terms of another, all correlation coefficients were in this predicted direction. The strongest and most consistent correlations were found between psychological performance and the degree of change within large cortical neurons, namely cell loss and reductions in nuclear and nucleolar volume and cytoplasmic RNA content. Other neuropathological measures, the frequency of senile plaques and neurofibrillary tangles showed only weak correlations with psychological measures, coefficients tending nevertheless to be higher for tangle than for plaque frequency. The degree of reduction in ACh synthesis was significantly correlated with the extent of cognitive impairment as indexed by the clinical rating scale and visual reaction time test performance, but not with other measures of psychological performance. Reduction in ACh synthesis correlated also with nuclear and nucleolar volume in layer V and with measures of cell loss, but not with the frequency of senile plaques and neurofibrillary tangles. In contrast, the extent of reduction of CAT activity correlated with both plaque and tangle frequency, the correlation with plaques being the stronger. CAT activity showed no significant correlation

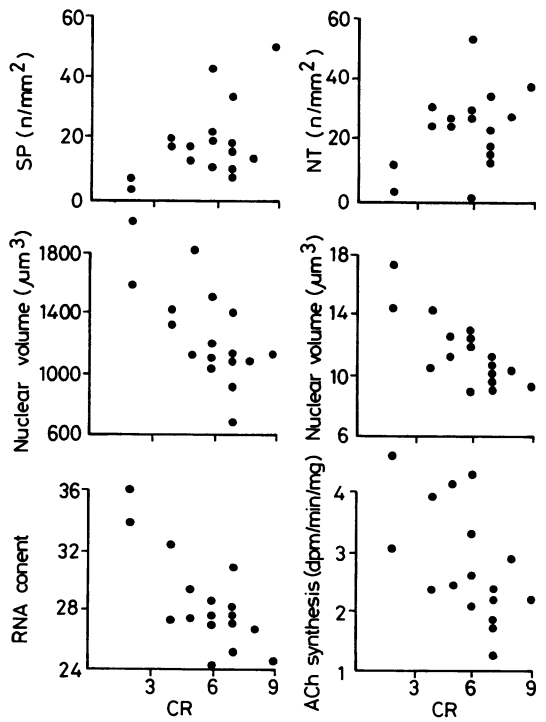

Fig 1 *Relationship between severity of dementia, measured by the Clinical Rating, and pathological and chemical indices of disorder in Alzheimer's disease. CR = Clinical rating. SP = senile plaques, NT = neurofibrillary tangles: correlations with CR are not statistically significant. Nuclear and nucleolar volume and RNA content represent values for layer V: correlations are significant at $p < 0·01$, $p < 0·001$ and $p < 0·01$ respectively. The correlation between ACh synthesis and CR is significant at $p < 0·01$.*

Table 8 *Psychological-chemical correlations*

	Clinical rating of severity	*WAIS*		*Token test*	*Visual reaction time*
		Verbal	*Performance*		
CAT activity	−0·24	0·08	0·05	0·08	−0·29
ACh synthesis	−0·63†	0·12	0·31	0·16	−0·45*

Spearman's (r) rank correlation.
† = $p < 0.01$; * = $p < 0.05$.
Low chemical values, and low WAIS and token test scores represent high impairment; low values on the clinical rating scale and reaction time test represent low impairment, hence the inverse correlations.

however with the degree of cognitive impairment, nor with measures of perikaryal function.

The weak relationship demonstrated in the present study between neurofibrillary tangle frequency and severity of dementia contrasts with the highly significant correlation between tangle count in the temporal lobe and degree of dementia reported by others in elderly Alzheimer's disease patients.[11] It is noteworthy however that in this latter study statistical analysis included non-demented patients, in whom neurofibrillary tangles were absent. Higher levels of significance would therefore be expected, since they reflect the fact that the *presence* of dementia is associated with the *presence* of neurofibrillary tangles, and are not simply a measure of the relationship between *severity* of tangles and dementia.

The lack of evidence for a strong relationship between plaque frequency and severity of dementia observed in the present study confirms previous reports derived from necropsy studies.[11 19 20] It has been suggested[9] that a threshold effect may underlie the finding of a non-significant relationship, whereby increases in severity of dementia may cease to be detected despite continuing increases in plaque formation. Whilst this argument might apply to necropsy studies, in which dementia is likely to be advanced at the time of clinical evaluation of patients, it can less easily account for the present findings, since the study group encompassed a wide range of patient severity. Moreover, a threshold effect in the clinical measurement of dementia, resulting in poor discrimination between patients, would be expected also to affect correlations with "perikaryal" measures where strong and consistent relationships were found.

The presence of "perikaryal" changes in Alzheimer's disease has been reported previously.[14] The significant correlations between decreases in pyramidal cell frequency and reductions in perikaryal changes, and between these features and degree of dementia indicate that both neuropathological and neuropsychological measures accurately reflect a failure of neocortical function. The strong correlation demonstrated between reduction in cytoplasmic RNA and frequency of neurofibrillary tangles in large cortical neurons reinforces this point. These findings therefore point to a disorder of large cortical neurons, and are in keeping with the specific reduction of large cortical neurons found in senile forms of AD by others.[21–23] Given that neurofibrillary tangles represent a defect within large cortical neurons it might be anticipated that tangle frequency would be more strongly related to "perikaryal" disorder than would senile plaque frequency. Some support for this prediction was demonstrated. Previous studies of the relationship between large cortical neurons and plaque frequency revealed no significant correlation between the latter and cortical cell counts or cortical thickness.[21]

It might be argued that the abnormalities within cortical neurons in Alzheimer's disease are simply secondary manifestations of impaired afferent pathways from areas such as basal forebrain nuclei, locus coeruleus and raphe nuclei (see Mann *et al*[24] for review). If this were so then a strong relationship might have been predicted between changes in nuclear volume and changes in both nucleolar volume and RNA content, the three measures simply reflecting a generalised slowing of metabolic processes stemming from failing synaptic contacts. However, weak relationships only were demonstrated, suggesting that a decreased nuclear volume, and by implication a reduced mRNA output from the nucleus may precede, or proceed independently from, the changes in nucleolar volume and cytoplasmic RNA content.

The reductions in ACh synthesis and CAT activity in the present study probably reflect a functional loss of cholinergic nerve endings in the cortex and are in keeping with the reports of reductions in choline uptake.[25 26]

If structural loss of cholinergic synapses results in a proportional loss of CAT apoenzyme, then a significant correlation between an index of structural synaptic change, (that is senile plaque frequency) might be expected to occur. If however, remaining neurons are capable of compensatory increases in the rate of ACh synthesis, then a similar proportional relationship between plaques might not obtain. In accordance with these predictions a significant cor-

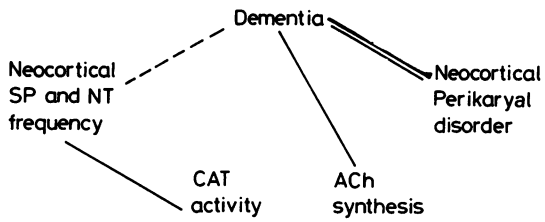

Fig 2 *Relationship between psychological, pathological and chemical disorder in Alzheimer's disease. Relative strength of relationship is indicated: by ═══ = strong; —— = moderate; - - - - = weak. SP = senile plaques; NT = neurofibrillary tangles; CAT = choline acetyltransferase; ACh = acetylcholine.*

relation was demonstrated between the reduction in CAT activity and frequency of senile plaques, but not between plaque frequency and reduction in ACh synthesis. A correlation between CAT activity and plaques has been reported before,[1027] but has not invariably been demonstrated.[12] Correlations between chemical, pathological and psychological measures indicated stronger relationships between ACh synthesis and other measures than CAT activity. Measures of ACh synthesis would then appear to be a more sensitive index of the physiologically active pool of ACh in the cortex than are measures of CAT activity. This may be because assays of ACh synthesis at high potassium ion concentrations actually reflect the release of neurotransmitter from synaptic nerve endings.[7]

Given the absence of a strong and consistent relationship between cholinergic markers and the changes in large cortical neurons (that is neurofibrillary tangle frequency and perikaryal disorder), it cannot be argued that the cortical changes of Alzheimer's disease are necessarily secondary to a primary subcortical disorder. Moreover, the possibility exists that subcortical change is secondary and retrograde to primary changes in the cortex. However, ACh is not the only major neurotransmitter known to be implicated in Alzheimer's disease.[8] Both noradrenergic and serotonergic failure[28] may contribute cumulatively to cortical cellular dysfunction. The principal relationships demonstrated by the present study are illustrated in fig 2.

In interpreting the findings it is important to recognise that less than one third of neocortical cholinergic activity is believed to occur in cortical interneurons.[29] The reduction in presynaptic cholinergic activity found in this study is therefore more likely to reflect retrograde degeneration of ascending cholinergic tracts,[30] or a failure of cholinergic cells in the nucleus basalis.[624] Since senile plaques also probably reflect changes in the synaptic endings of neurons arising within and projecting from the nucleus basalis[31] a significant correlation between the reduction in CAT activity and the frequency of senile plaques might have been anticipated.

The concept of a failure of subcortical systems utilising specific neurotransmitters and underlying the cortical abnormalities of Alzheimer's disease is an attractive one[8] because of its therapeutic implications. However, the finding in the present study of a strong correlation between the degree of dementia and the degenerative changes in large cortical neurons suggests that important abnormalities are present in the cortex as well as the subcortex, and the relationship between the two may be primary, secondary or may co-exist in parallel. It is of interest, for example, that in addition to the loss of large cortical neurons, significant loss of large neurons in the nucleus basalis, locus coeruleus and raphe has been observed,[624] and furthermore that the presence of neurofibrillary tangles was observed within these subcortical cells. The possibility exists therefore that a fundamental and possibly common abnormality in large cortical and subcortical cells underlies the pathogenesis of Alzheimer's disease. The characterisation of such neurons must be the task of further studies.

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