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Neocortical Morphometry and Cholinergic Neurochemistry in Pick's Disease

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With a computerized image-analysis apparatus for neocortical morphometry and chemical methods for evaluation of the cholinergic system, five brain specimens of Pick's disease (PD) were studied and the results compared to those from specimens of age-matched normal subjects and Alzheimer's disease (AD). The PD specimens showed major reductions in brain weight, frontal and temporal cortical thickness, and large neuron populations, compared with controls. Lesser reductions were seen in small neurons and thickness of the inferior parietal cortex. The authors found no relationship between age of onset or disease duration and either the degree of cortical thinning or neuron loss or the number of Pick bodies in the neocortex and hippocampus. PD specimens were more atrophic than AD brains, having lower brain weights and more frontotemporal thinning. Large neurons were comparably reduced in the two conditions in the frontal and temporal lobes, but small neuron losses were greater in the PD midfrontal area. Only the AD cases showed loss of large neurons in the inferior parietal region. Levels of choline acetyltransferase were normal in PD and reduced in AD, whereas muscarinic receptor binding was decreased in both. (Am J Pathol 1988, 131:507–518)

PICK'S DISEASE (PD) is an uncommon cause of dementia occurring in middle age and later life. Owing largely to its rarity, the neocortical atrophy and neuronal depopulation occurring in PD have thus far escaped quantification. The few neurochemical studies of Pick cerebral cortex have been restricted to only a few cases. Because Alzheimer's disease (AD) is many times more prevalent than PD, it has been the subject of numerous morphometric and neurochemical analyses. We have, in the course of compiling hundreds of cases of AD over the past several years, encountered five typical cases of PD. Using a computerized image analysis apparatus and quantitative chemical methods, these specimens have been characterized both morphometrically and neurochemically. Many brains from age-matched mentally normal individuals have been similarly processed to serve as controls. We are now able to compare morphometric and cholinergic neurochemical data from Pick cerebral cortex with that obtained both from normal controls and from AD patients of comparable ages.

The comparisons between Pick and normals should serve further to characterize the disease anatomically and in terms of the cholinergic system. The comparisons between PD and AD, both characterized by neocortical neuronal loss and gliosis, will illuminate differences beyond those traditionally invoked; that is, lobar atrophy and Pick bodies in the former and generalized atrophy accompanied by plaques and tangles in the latter. This type of analysis also demonstrates previously unsuspected similarities in these neurodegenerative conditions.

Materials and Methods

Cases Studied

The 5 patients with PD included 3 women, aged 67, 82, and 84, and 2 men, aged 65 and 72. All bore clini-

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Table 1—Cases Studied

Case	Age	Sex	Disease duration (y)	Brain weight (g)	Gross atrophy	Neuron loss and gliosis	Pick bodies
S.V.	65	Male	12	1030	F, T, A, H	F, T, H	F, T, P, H, BS
J.B.	67	Female	10	824	F	F, T, P, A	F, T, P, A, H, BG, HY, BS
J.R.	72	Male	7	982	General	F, T, A	F, T, P, A, H
M.W.	82	Female	16	632	F, T, C, A, H	F, T, A, H	T, H
A.P.	84	Male	16	985	F, T, A, H	F, T, P, A, H	F, T, P, A, H

F, frontal; T, temporal; P, parietal; C, caudate; A, amygdala; H, hippocampus; Hy, hypothalamus; BG, basal ganglia; BS, brain stem.

cal diagnoses of AD or senile dementia of the Alzheimer type with histories of progressive intellectual deterioration extending over many years and terminating in profound dementia. Gross neuropathologic evaluation revealed frontotemporal lobar atrophy in 3 cases, frontal atrophy alone in 1, and a single instance of generalized atrophy with ventral temporal accentuation. Microscopic examination utilizing Sevier–Munger silver stains revealed numerous hippocampal and neocortical Pick bodies in 4 of the 5 cases. In one instance of frontotemporal lobar atrophy (an 82-year-old woman), only a few Pick bodies were found despite severe neuronal loss and gliosis. The neuropathology of these five specimens is summarized in Table 1.

The control comparison group for the neocortical morphometry included 27 normal specimens from individuals selected to range in age from 65 to 84. These patients were either evaluated clinically and considered to be without evidence of dementia, or they came from walks of life which would be inconsistent with significantly impaired mentation. The brains were examined grossly and microscopically (detailed below) and found to be normal.

The AD comparison group comprised 80 individuals between 65 and 84 years of age. These patients were all clinically demented. The diagnosis of AD or SDAT was confirmed microscopically by the identification of numerous neocortical neuritic plaques and neurofibrillary tangles. In a few cases involving the more elderly patients, neocortical plaques were unaccompanied by tangles.[1] Not all of the cases in the various comparison groups were studied as to all of the parameters under discussion.

Specimen Processing

All brains were removed in the traditional fashion within 24 hours of death. Each was then divided sagitally in the midline; then the left hemibrain was immersed in 10% buffered formalin, and the right was frozen at −70 C. After 7–10 days of fixation, the left hemibrain was weighed, and the fixed whole brain weight was derived by doubling the hemibrain value. For purposes of neocortical morphometry, blocks of tissue were taken from the left midfrontal region (Brodman area 9, 10, or 46), superior temporal gyrus (area 38), and the inferior parietal (area 39 or 40) lobule. Routine neuropathologic evaluation entailed examining sections stained with hematoxylin and eosin (H&E) from these neocortex blocks as well as sections from the hippocampus, amygdala, substantia innominata, cingulate gyrus, visual cortex, mesencephalon, pons, and cerebellum. Ten-micron sections of these blocks were stained with 1% thioflavin S and viewed with ultraviolet light through flourescein isothiocyanate filters for detection of neuritic plaques and neurofibrillary tangles. Sevier–Munger silver stains were employed for the further identification and quantification of Pick bodies, although most were visible with the H&E preparations.

Neocortical Morphometry

The method utilized for neocortical morphometry and the techniques for manual editing of the video image have been previously detailed.[2] Cortical thickness and cell counts were determined on 20-μ-thick cresyl violet preparations from the midfrontal, superior temporal, and inferior parietal tissue blocks. The counted area measured 600 μ along the pial surface on the side of the gyrus and extended through the full cortical thickness. The greater optical density of cell bodies as opposed to neuropil allowed detection and measurement of the perikarya. These were assigned to ten size classes on the basis of cross-sectional area: 5–30 sq μ, 31–40 sq μ, 41–60 sq μ, 61–90 sq μ, 91–140 sq μ, 141–200 sq μ, 201–300 sq μ, 301–500 sq μ, 501–800 sq μ, and greater than 800 sq μ. Comparisons were made between the cells as they appeared in the microscope and the various size categories into which they fell when their video images were measured by the computer. It was shown previously that in the necortex of Alzheimer patients and of normal controls, the

great majority of cells of cross-sectional area 40 sq μ and less are glia, while nearly all of those greater than 40 sq μ are neurons.[2] For the purpose of facilitating comparisons, the ten size classes have been summarized into three: cells less than 40 sq μ as glia, cells between 40 and 90 sq μ as small neurons, and cells greater than 90 sq μ as medium and large neurons.

The situation in PD is more complicated than in normal or Alzheimer specimens because many of the enlarged reactive fibrous astrocytes in the Pick neocortex fall into the 40–90-sq μ size class. We directly examined 223 cells in the 40–90-sq μ category from the neocortex of a representative Pick case. Up to 33% of these cells could be identified as glia, predominantly fibrous astrocytes. Only 39% could be reliably designated as small neurons. The remaining 28% could not be confidently identified specifically as either neurons or glia on the basis of their appearance in the cresyl violet preparation. The criteria employed for the identification of neurons and glia in the cresyl violet sections were morphologic. Specifically, cells that were obviously neurons usually had round nuclei with prominent centrally positioned nucleoli, a pyramidal shape, and Nissl substance in the cytoplasm. The glial cells had small, darkly stained nuclei without discernible cytoplasm in the case of oligodendroglia, and larger, pale nuclei with imperceptible or tiny nucleoli when astrocytic in character. Reactive astrocytes had ample homogeneous or fibrous cytoplasm and irregular shape. Most of the glia–neuron overlapping occurred in the 40–60 sq μ category, where 37% of the cells were glia. In the 60–90 sq μ category, only 19% were glia and 65% were neurons, while 17% could not be identified with certainty. The problems raised by this fibrous astrocyte contamination of what is in normals and AD the small neuron (40–90 sq μ) category is further dealt with in the section on results.

In the early years of this study, the instrument used for morphometry was the Quantimet 720. This was later replaced by the Quantimet 920, but the two instruments yield consistent results when applied to the same slides. The two-tailed Student t test was used for comparing morphometric data from the Pick cases with those derived from normal subjects and AD cases.

Quantification of Pick Bodies

In order to determine whether the neocortical morphometric parameters were directly related to the pathognomonic inclusions of PD, we also quantified Pick bodies in the midfrontal, superior temporal, and inferior parietal regions. The cortical areas assayed measured 6 mm along the pial surface on the side of the gyrus and extended through the full cortical thickness. In most instances the neocortex in which these inclusions were counted overlapped the regions measured by the Quantimet. The Pick bodies were darkly stained by the Sevier–Munger silver stain technique on 6-μ-thick sections, and manual counting was performed on a Leitz microscope. Concentrations of hippocampal Pick bodies were evaluated semiquantitatively.

Neurochemistry

Neurochemical analyses were performed on samples of frozen right hemispheres dissected on a glass plate over a bed of powdered dry ice.[3] The control group for neurochemical analysis comprised 5 clinically and neuropathologically normal individuals between the ages of 59 and 75. Frozen tissue was available for analysis from 4 of our 5 Pick cases. Twenty-five cases from AD patients between 65 and 82 years of age were used as a third comparison group. Tissues assayed for choline acetyltransferase activity and muscarinic receptor binding included midfrontal, superior temporal, inferior parietal, medial orbital, and entorhinal cortices, substantia innominata, substantia nigra, caudate nucleus, raphe nucleus, accumbens nucleus, amygdala, and hippocampus. Choline acetyltransferase activity was measured by the method of Fonnum[4] as modified by Davies.[5]

Total muscarinic receptor binding was estimated with ^{3}H-N-methyl scopolamine (New England Nuclear, 70–87 Ci/mmol) by a filtration method (P. Davies and I. Creese, personal communications). Tissues were homogenized in 1 ml cold 50 mM Tris HCl (pH 7.4), and the homogenates were washed by the addition of 9 ml buffer, vortexing, and centrifuging at 17210g for 30 minutes. Samples were then decanted, and the pellets were suspended in 10 ml buffer. A Lowry total protein[6] determination was performed, and all samples were diluted to a standard protein concentration of 0.1 mg/ml prior to assay. It should be noted that this protein value represents mainly the particulate, noncytosol fraction of the homogenate. Aliquots were incubated in triplicate tubes (1.0 pmol ligand per tube, final volume of 1 ml) for 90 minutes at room temperature. Total and nonspecific binding tubes were run in parallel and were identical except for the addition to the latter of 10 μl of 0.1 mM atropine sulfate as a specific binding inhibitor. Separation of bound from unbound ligand was accomplished by rapid vacuum filtration of the incubation mixture through Whatman glass fiber filters (Whatman GF/

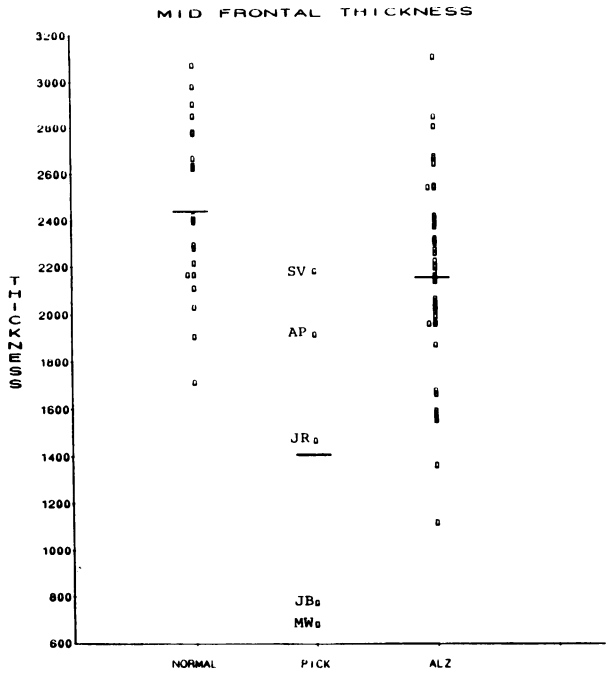

Figure 1—Units are microns; dark lines are means. Standard errors of means: normals, 79; Pick, 334; Alzheimer, 68.

C) followed by two 5-ml washes with Tris buffer. The radioactivity of bound ligand for each sample was then determined by scintillation spectrophotometry. Specific receptor binding was calculated as the difference between the average total and nonspecific binding measurements and expressed in picomoles per 100 mg of total protein. Neurochemical data from the three groups (normals, PD, and AD) were compared with one another with the use of one-way analysis of variance and the Student *t* test.

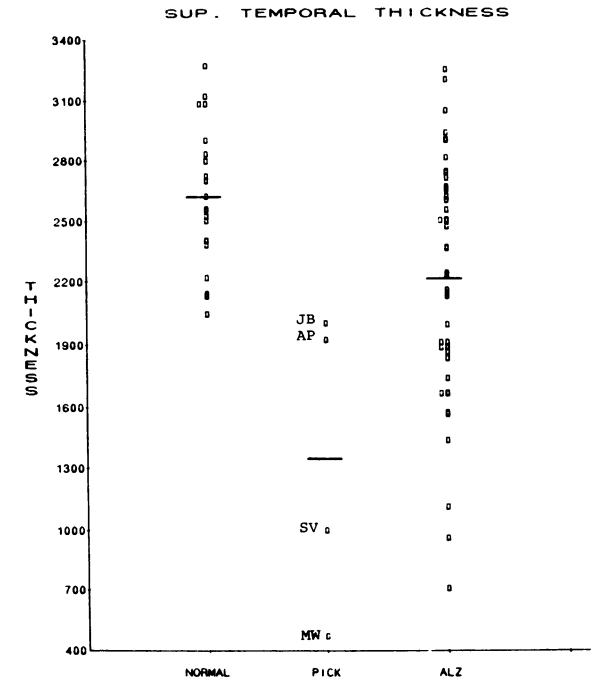

Figure 2—Units are microns; dark lines are means. Standard errors of means: normals, 78; Pick, 428; Alzheimer, 86.

Results

Neocortical Morphometry of PD Versus Normals

The morphometric data from Pick cerebral cortex, when compared with that from age-matched controls, serve largely to quantify long-standing observtions concerning the severity and distribution of neocortical atrophy in this condition. Whole brain weight is severely diminished, averaging 891 g in PD and 1245 g in the controls ($P < 0.001$). Cortical thickness is reduced by 42% in the frontal lobe ($P < 0.001$, Figure 1) and by 49% in the temporal ($P < 0.001$, Figure 2), but only by 22% in the inferior parietal area ($P < 0.05$). In the midfrontal and superior temporal regions, total cell populations are decreased by 37% and 39%, respectively ($P < 0.01$), but no such loss is detectable in the inferior parietal area.

In the control brains, almost all the cells in the 40–90-sq μ size class are small neurons, but, as discussed in the Materials and Methods section, this is not the case in the Pick cortex, where this size category is heavily contaminated by the presence of enlarged fibrous astrocytes. Comparing populations of cells between 40 and 90 sq μ in the control group with those in the Pick group, therefore, underestimates the loss of small neurons, because some of these cells in the Pick cortex are in fact not small neurons, but rather, large astrocytes. Nevertheless, in the midfrontal cortex, cells between 40 and 90 sq μ are reduced by 30% in PD. This 30% loss as detected by our counting method represents a minimum value as to neurons, and the actual loss of neurons in this size category is undoubtedly higher. In the superior temporal gyrus, although there is no statistically significant loss of cells in the 40–90-sq μ class as a whole, in the 60–90-sq μ subcategory (which is less distorted by the presence of fibrous astrocytes), there is a significant ($P < 0.02$) reduction in cells, amounting to 38%. This again represents a minimum value, the actual loss of small neurons being undoubtedly greater.

Both the midfrontal and superior temporal areas in PD show extensive loss of medium and large neurons (cells greater than 90 sq μ). In the former, such neurons are reduced by 56% ($P < 0.01$, Figure 3), and in the latter, the decrement is 63% ($P < 0.001$, Figure

4). The percentage reductions increase with the size categories; that is, the Pick midfrontal cortex has 50% fewer neurons 91–140 sq μ, 57% fewer 141–200 sq μ, 72% fewer 201–300 sq μ, etc. A similar pattern occurs in the superior temporal gyrus.

Despite its cortical thinning, the inferior parietal lobe does not participate in this neuronal depopulation. Here there are no significant differences in cell counts in any of the size categories when the Pick group is compared with the normals. As would be expected in light of cortical thinning with unchanged neuron counts, the Pick group has a higher density of neurons than the control group. The increase is 43%, and the P value is less than 0.02 for this parameter. Neuronal density is calculated by dividing the number of cells greater than 40 sq μ by the total area counted. Because some fibrous astrocytes are included in the greater than 40 sq μ class, this calculated increase may be somewhat exaggerated. Fibrous astrocytes are, however, less prominent in the inferior parietal lobe than in the frontal and temporal regions, but only in the parietal is this density parameter significantly increased in PD.

Individual values for cases can also be compared with the mean values for the control group. This analysis shows that there is not a simple correlation between age of onset or disease duration and the degree of cerebral atrophy as measured by cortical thinning and neuronal loss (Table 1, Figures 1–4). For example, Patient S.V. was 53 when symptoms began, and his course ran for 12 years. His individual values for cortical thickness and large neuron populations in the midfrontal cortex fall near 1 standard deviation from the control group average, while the same parameters in Patient J.B. (who was 57 at onset and survived for 10 years) fall outside three standard deviations. Between these extremes are durations both longer (A.P., 16 years) and shorter (J.R., 7 years).

Neocortical Morphometry of Alzheimer Disease Versus Normals

Several morphometric analyses of the cerebral cortex in AD have been previously published.[2,7,8] This neocortical morphometry is briefly explored again only so that the severity and distribution of neuronal loss in AD can later be compared with that occurring in PD.

Whole brain weight is diminished (<0.001) in AD. Cortical thickness is reduced in the frontal, temporal, and parietal lobes by 13–19% ($P < 0.01$, Figures 1 and 2). Total cell populations are decreased in the mid-

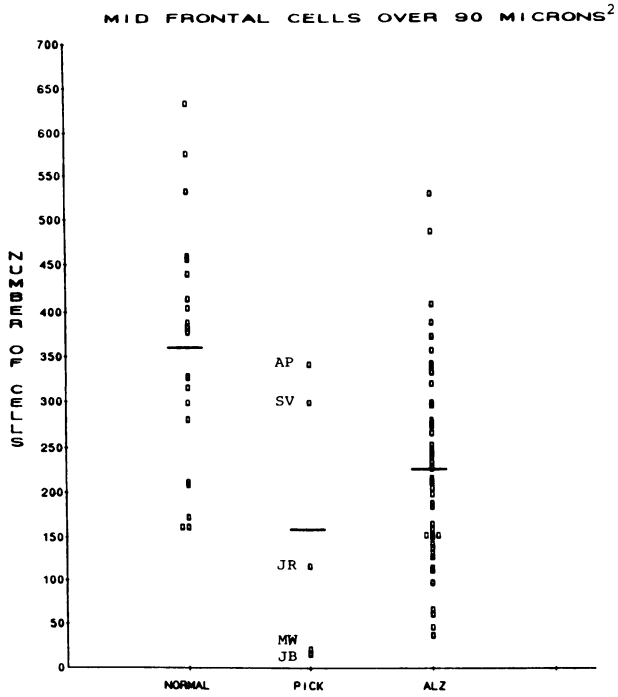

Figure 3—Cells > 90 sq μ are medium and large neurons. Dark lines are means. Standard errors of means: normals, 28; Pick, 77; Alzheimer, 15.

frontal ($P < 0.02$) and superior temporal ($P < 0.01$) regions, and all three neocortical areas have lost significant numbers of neurons.

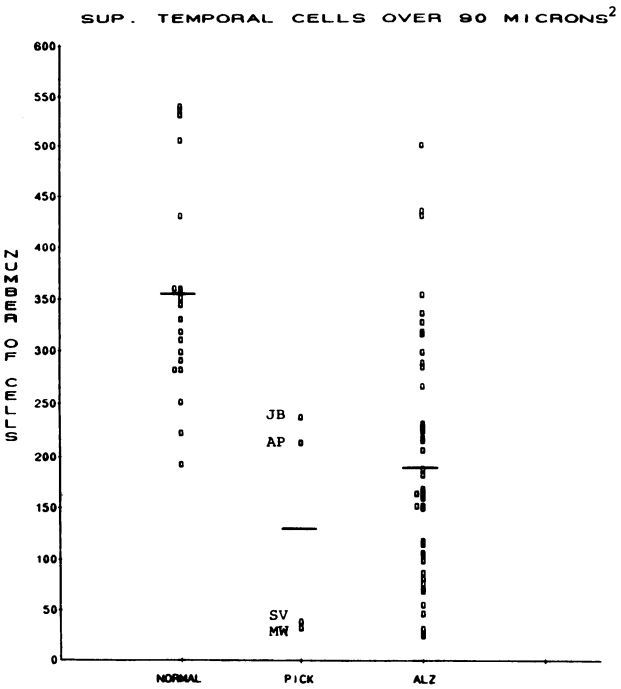

Figure 4—Cells > 90 sq μ are medium and large neurons. Dark lines are means. Standard errors of means: normals, 23; Pick, 64; Alzheimer, 17.

Small neurons (cells 40–90 sq μ) are reduced in AD, compared with controls, only in the superior temporal gyrus, where there is a loss of 15% ($P < 0.05$). Small fibrous astrocytes are increased in AD, compared with controls,[9] and this could theoretically reintroduce the type of error (specifically, underestimation of small neuron losses) which was considered when comparing Pick cortex with controls. In practical terms, however, the reported increases in fibrous astrocytes in the Alzheimer cortex, while statistically significant, are small individually and in terms of absolute numbers. In fact, their population is less than 10% of the total cell count in the 40–90-sq μ size category and therefore cannot be expected to alter either the level of statistical significance or percent reduction of small neurons.

All three cortical regions show highly significant ($P < 0.001$) losses of medium and large neurons (Figures 3 and 4). The percent reductions are 37% in the midfrontal, 46% in the superior temporal, and 45% in the inferior parietal.

Neocortical Morphometry of Pick Versus Alzheimer Disease

As described above, both PD and AD cause significant cortical atrophy and neuronal loss, which is more severe in the larger neuron size classes. The diseases differ from one another primarily in the distribution of the neuronal depopulation and its severity. PD brains are more atrophic than those in AD, as reflected by whole brain weights ($P < 0.002$). There is also significantly greater cortical thinning in the frontal and temporal lobes in PD ($P < 0.01$, Figures 1 and 2). In the midfrontal cortex, PD brains have at least 26% fewer small neurons (40–90 sq μ) than have AD brains ($P < 0.05$), but the actual percentage reduction is probably greater because of the large astrocytes as detailed above. Significant differences were not found in small neuron populations in the superior temporal gyrus, since a slight decrease occurs there in AD; and the loss of such cells in PD is, again, artifactually minimized by the fibrous astrocytes. No differences were seen in small neuron populations in the inferior parietal area. AD specimens have fewer large neurons in the inferior parietal cortex ($P < 0.02$) than in PD, but cortical thicknesses are similar. Large neuron populations in the midfrontal and superior temporal areas do not differ significantly in the two diseases (Figures 3 and 4).

Quantification of Pick Bodies

The results of counting Pick bodies are presented in Table 2. In those cases where Pick bodies were found in large numbers (Cases J.R., J.B., and A.P.), they could be seen in all the cellular layers of the cortex, but they often seemed particularly numerous in Layer 2. The inclusions could be found in neurons from all size categories. No significant correlations were found between Pick body counts and age of onset, disease duration, cortical thickness, or degree of neuronal loss in any of the three neocortical regions measured (Table 2, Figures 1–4).

We also semiquantitatively evaluated Pick bodies on single sections of hippocampus. They were found literally by the hundreds in both the dentate fasciculus and the pyramidal cell layer in 3 of the 5 cases (J.B., J.R., and A.P.). In patient S.V., dozens of inclusions were found in the dentate, and many were seen in pyramidal cells; but in terms of total numbers, Pick bodies were far less numerous in this patient than in the three indicated above. In the case of M.W., hippocampal atrophy was so severe that the granular cells had largely disappeared, and Pick bodies could not be identified there with certainty. Only scattered inclusions were found in the comparatively better preserved but still significantly depopulated pyramidal cell layer.

When the results in Table 2 are examined, one sees at least a rough correspondence between numbers of hippocampal and neocortical Pick bodies. That is, those patients with hundreds of hippocampal Pick bodies also have the highest counts in the neocortex (J.B., J.R., and A.P.), while the patient with the fewest hippocampal Pick bodies (M.W.) has only rare neocortical ones. In the case of patient S.V., both hippocampal and neocortical Pick body concentrations occupy a middle ground between the extremes. In all cases, Pick bodies were more concentrated in the hippocampus than in the neocortex. One sees again in the hippocampus as in the neocortex that there is no apparent relationship between concentrations of Pick bodies and age of onset, disease duration, or degree of cerebral atrophy.

Neurochemistry Results

Significant differences ($P > 0.05$) were not found in choline acetyltransferase activity in any of the brain regions assayed when the Pick cases and controls were compared (Figure 5). However, ChAT activity was significantly reduced in the Alzheimer group, compared with controls in the midfrontal ($P < 0.01$), superior temporal ($P < 0.001$), and inferior parietal ($P < 0.02$) areas of neocortex, as well as in the hippocampus ($P < 0.05$) and entorhinal cortex ($P < 0.001$, Figure 5). Accordingly, these differences can also be ex-

Table 2—Pick Body Counts

Case	Midfrontal	Superior temporal	Inferior parietal	Hippocampus	
				Granular layer	Pyramidal layer
S.V.	5	5	1	Dozens	Dozens
J.B.	57	317	203	Hundreds	Hundreds
J.R.	265	NA	40	Hundreds	Hundreds
M.W.	1	3	0	None	Few
A.P.	18	106	20	Hundreds	Hundreds

The cortex in which Pick bodies were quantified measured 6 mm along the pial surface and extended through the full cortical thickness. Pick bodies in the hippocampus were evaluated on single sections from each case.

pressed by comparing ChAT levels in the Pick group with those in the Alzheimer group, with the Pick values being significantly higher in the neocortex (M.F., $P < 0.001$; S.T., $P < 0.001$; I.P., $P < 0.05$).

Muscarinic receptor binding using the ligand ^{3}H-NMS was reduced in Pick disease brain, compared with controls in the midfrontal ($P < 0.01$), superior temporal ($P < 0.001$), inferior parietal ($P < 0.01$), and entorhinal ($P < 0.05$) cortex (Figure 6). In comparisons of Alzheimer versus controls, however, significant reductions in muscarinic receptor binding were found only in the midfrontal, superior temporal, and entorhinal regions, all at $P < 0.05$. Other assayed areas showed variable percentage reductions in receptor binding in the Alzheimer brains when compared with controls, but these did not attain statistical significance.

Discussion

Opinions vary concerning those neuropathologic features requisite for the diagnosis of PD, but when gross frontotemporal lobar atrophy is accompanied microscopically by argentophilic Pick bodies, all observers would concur with this designation. Most authorities would also agree that the presence of Pick bodies, even in the absence of classic frontotemporal atrophy, would warrant such a diagnosis. Some authors stress the significance of swollen, chromatolytic neurons in this entity, ascribing to them the same importance as the Pick body.[10] We were able to identify that sort of cell in 3 of our 5 Pick's cases (J.R., M.W., and J.B.), and although they could be seen with the H&E stains, they were more prominent in the Nissl preparations and with silver stains. Since the designation of a particular cell as "swollen" and "chromatolytic" is more subjective than either computerized measurement of a cell or the identification of a distinctive argyrophilic inclusion, we did not attempt to quantify such cells in the Pick brains. European neuropathologists may designate a case as Pick's disease solely on the basis of lobar atrophy alone despite the

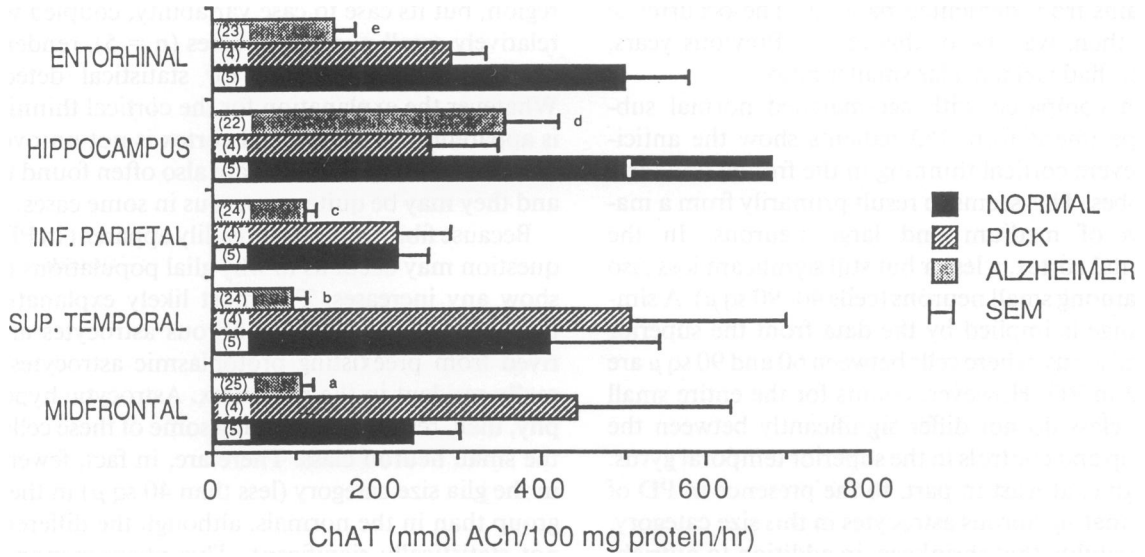

Figure 5—ChAT activity in normal (*solid bars*), Pick (*striped bars*), and Alzheimer (*gray bars*) brains. Enzyme activity is expressed as nanomoles acetylcholine synthesized per 100 mg protein per hour. *a*, Alzheimer versus normal ($P < 0.01$) and Alzheimer versus Pick ($P < 0.001$); *b*, Alzheimer versus normal and Alzheimer versus Pick ($P < 0.001$); *c*, Alzheimer versus normal ($P < 0.02$) and Alzheimer versus Pick ($P < 0.05$); *d*, Alzheimer versus normal ($P < 0.05$); *e*, Alzheimer versus normal ($P < 0.001$).

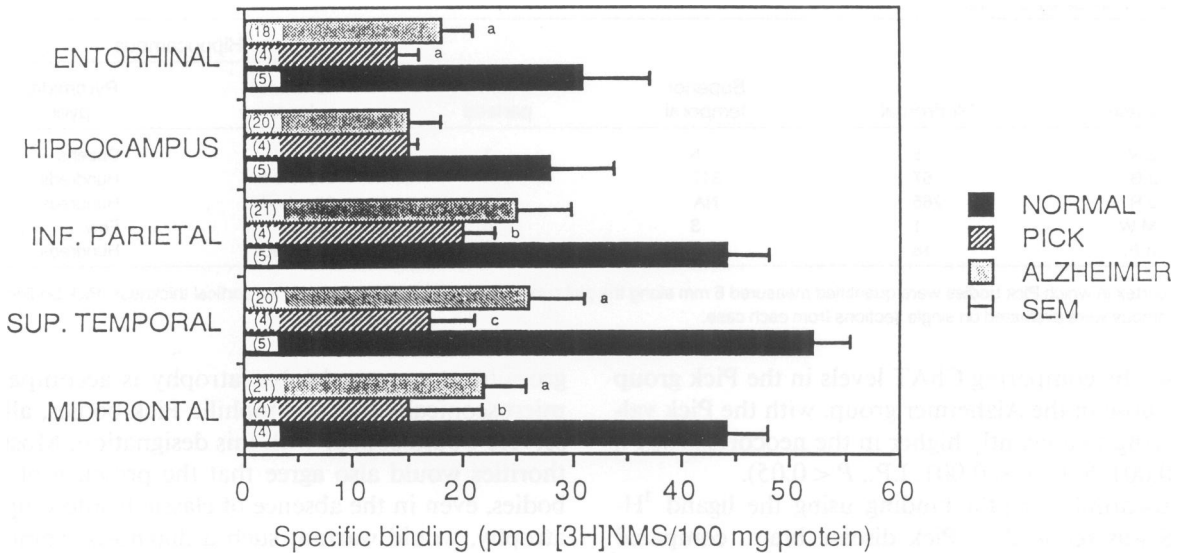

Figure 6—Muscarinic receptor binding in normal (*solid bars*), Pick (*striped bars*), and Alzheimer (*gray bars*) brains. Receptor binding is expressed as picomoles ^{3}H-N-methylscopolamine bound per 100 mg proten. *a*, Alzheimer or Pick versus normal ($P < 0.05$); *b*, Pick versus normal ($P < 0.01$); *c*, Pick versus normal ($P < 0.001$).

absence of any specific neuronal abnormalities.[11] It has even been contended that PD and AD represent points along a continuous spectrum of cortical neuronal degeneration.[12] The morphometric findings of our study are derived only from cases in which unequivocal Pick bodies could be found.

The five examples of PD presented in this report had all been diagnosed clinically as probable AD. They were sent to us between March 1985 and September 1986, during which time we received a total of 165 brains from demented patients. The occurrence of PD, then, was 3% of this group. Previous years, however, had yielded a far smaller ratio.

When compared with age-matched normal subjects, specimens from PD patients show the anticipated severe cortical thinning in the frontal and temporal lobes. This seems to result primarily from a major loss of medium and large neurons. In the midfrontal cortex, a lesser but still significant loss also occurs among small neurons (cells 40–90 sq μ). A similar change is implied by the data from the superior temporal gyrus, where cells between 60 and 90 sq μ are reduced in PD. However, counts for the entire small neuron class do not differ significantly between the PD group and controls in the superior temporal gyrus. This is due, at least in part, to the presence in PD of contaminating fibrous astrocytes in this size category. The possibility that shrinkage, in addition to outright neuronal loss, occurs among medium and large neurons should also be considered. This would place the shrunken neurons in smaller size categories and would be consistent with the observed increasing percent reductions of neurons with increasing size categories. Unexpectedly, the parietal cortex is significantly thinner in the Pick cases than in normals, despite the absence of statistically important reductions in any of the neuronal size classes. This could represent loss or degeneration of corticocortical projecting pathways between the parietal lobe and the severely atrophic frontal and temporal lobes. Alternatively, neuronal loss may be occurring in the inferior parietal region, but its case to case variability, coupled with a relatively small number of cases (n = 5), renders the mean change too subtle for statistical detection. Whatever the explanation for the cortical thinning, it is apparent that the parietal cortex is not uninvolved in PD, because Pick bodies are also often found there, and they may be quite numerous in some cases.

Because fibrous gliosis is readily apparent in PD, the question may occur as to why glial populations fail to show any increases. The most likely explanation is that most of the enlarged fibrous astrocytes are derived from preexisting protoplasmic astrocytes normally resident in the neocortex. Astrocytic hypertrophy, then, results in displacing some of these cells into the small neuron class. There are, in fact, fewer cells in the glia size category (less than 40 sq μ) in the Pick group than in the normals, although the difference is not statistically significant. This phenomenon is at least partially attributable to the effects of cortical shrinkage in PD, which results in a far smaller total cortical area in the diseased brains. Supporting astro-

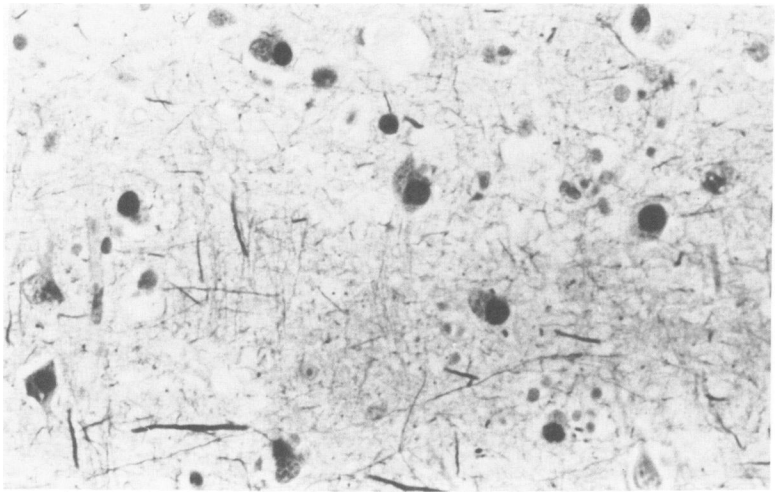

Figure 7—In cases with high neocortical Pick body counts, many inclusions were found in Lamina 2. (Patient J.R., Sevier–Munger stain, ×400)

cytes and oligodendroglia may also disappear along with their associated neurons and axons, so that total numbers of glia are reduced in PD despite an increase in fibrous astrocytes per unit area.

When the neuronal loss, gliosis, and consequent spongy rarefaction of the neuropil in PD do not devastate the entire cortical thickness, there appears to be a relative sparing of the deeper cortical layers. It would be of interest in this regard to relate the neuronal depopulation quantified in this study to the normal neocortical laminations. However, the atrophy in the Pick cases is so severe as to obscure the normal cytoarchitecture, and renders such an analysis impractical. It may be worth noting in this context that also in AD, as noted by Alzheimer himself in his original description,[13] neuronal loss is especially severe in the upper cell layers. Furthermore, recent quantitative studies in that disorder have shown that the majority of neuritic plaques occur in the upper cortical layers[14] even though mature plaques are more common in the deeper region.

The intracytoplasmic inclusions in PD and the neurofibrillary tangles in AD appear to be distributed differently in affected regions of neocortex. Tangles are found predominantly in Layers 3 and 5, and they show definite clustering.[14] Pick bodies, on the other hand, are often as numerous in Lamina 2 as in the deeper cortical layers (Figure 7). As this distribution would imply, Pick bodies can be found in neurons of all sizes, while tangles are more restricted to larger, pyramidal cells. The involvement of small neurons in PD has long been noted in the hippocampus, where many of the dentate neurons contain inclusions. In the Alzheimer hippocampus, tangles are essentially never seen in those granular layer cells, but rather inhabit the neurons of the pyramidal cell layer and subiculum.

Quantification of Pick bodies revealed a very wide case-to-case variation, but at least some of these inclusions had to be present in every case to assure the diagnosis and allow the specimen into this series. It has been suggested that the pathogenesis of PD involves a progression from an early stage, where presumably Pick bodies are numerous, to a later stage, where neuronal loss and gliosis predominate and Pick bodies are scarce.[15] Our data do not support this contention, because we could find no correlations between Pick body counts and either disease duration or extent of cortical thinning and neuronal loss. In some instances, Pick bodies could be readily identified in the midst of considerable gliosis, neuronal loss, and microcystic rarefaction (Figure 8). However, because by definition a Pick body must be intraneuronal, a total absence of neurons would necessitate a count of zero. This is not the case in AD, where tangles can be found as "ghosts" in the neuropil after containing neurons have disappeared.

In the midfrontal and superior temporal regions, neocortical morphometry of PD parallels that of AD. The frontal and temporal cortices are dramatically thinner in the Pick cases, but the larger neuron populations are comparably reduced in the two conditions. A greater loss of small neurons is seen in PD in the midfrontal cortex. The neocortical morphometry of the two diseases diverge more when the inferior parietal cortex is examined. There is no difference in cortical thickness here, with both thinner than controls. However, large neurons are reduced in Alzheimer inferior parietal cortex, compared with Pick.

Over the past several years, many studies have been published concerning the loss of large cholinergic neu-

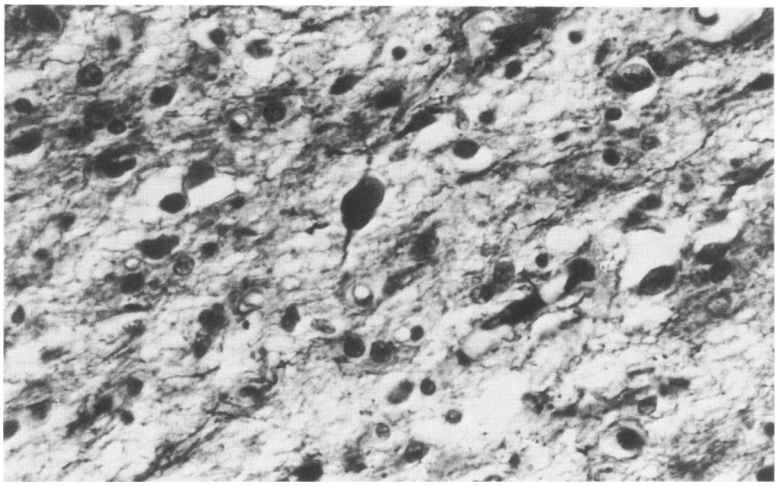

Figure 8—Pick bodies could often be identified in remaining neurons despite extensive surrounding gliosis, microcytic rarefaction, neuron loss, and cortical thinning. (Patient J.B., midfrontal cortex, Sevier–Munger stain, ×528)

rons from the nucleus basalis of Meynert (nbM) in AD.[16–19] In a few investigations, large neuron populations in the nbM have been quantified in small numbers of Pick cases.[20–22] Unlike AD, where nbM neuron loss has been found consistently, the results in PD have been variable. In one study of 5 Pick cases, there was no significant depopulation in the nbM.[20] A second investigation reported moderate loss occurring in 2 Pick patients.[21] A third study found an approximately two-thirds reduction in large nbM neurons in three Pick brains.[22] H&E and cresyl violet sections of the nbM were examined, but not quantified, in all 5 cases of PD evaluated in the present study. In 4 of the 5, large neuron populations were considered comparable to those seen in age-matched controls. In one instance, a slight reduction was perceived. Admittedly, quantification could conceivably reveal minor but statistically significant losses in this nucleus. Clearly, however, if such subtle depopulation has occurred, it pales in comparison with the profound neocortical neuronal loss documented above and with the major nbM loss in AD.

Absolute, or at least relative, preservation of the large cholinergic cortical projecting neurons in the nbM is to be anticipated in light of the normal neocortical ChAT activity which we found in the Pick brains. Choline acetyltransferase (ChAT) is a presynaptic cholinergic marker. Most previous investigations of neocortical ChAT activity in PD have yielded results similar to ours.[10,23–25] This preservation of ChAT activity in PD contrasts sharply with the significant reductions of neocortical ChAT which have been documented in AD.[5,26,27] Our Alzheimer comparison group also showed significant reductions in ChAT activity in the neocortex, as well as in entorhinal cortex and hippocampus.

We found significantly diminished muscarinic cholinergic receptor binding in PD, compared with controls, in the midfrontal, superior temporal, inferior parietal, and orbital frontal neocortex. Although muscarinic receptors have also been studied by others, there is no unanimity of opinion concerning their status in PD. White et al found that temporal lobe muscarinic receptor binding types were reduced to 47% of controls in PD.[28] Yates et al reported normal levels of muscarinic receptor binding in histologically normal regions of Pick brains, but decreased binding sites in those areas of neocortex with the neuropathologic features of PD.[23] Wood et al were unable to detect diminished muscarinic binding sites in their analysis of 4 cases.[25] All of these investigators used the muscarinic antagonist ^{3}H-quinuclidyl benzilate (^{3}H-QNB) as the ligand for measuring muscarinic receptor sites. We used another antagonist as ligand, ^{3}H-N-methylscopolamine (^{3}H-NMS), which has a different binding profile and is more hydrophilic than ^{3}H-QNB (I. Creese, personal communication).

Although not all investigators agree that muscarinic receptors are decreased in PD, the reductions we report in the present study correlate well with the loss of neurons in the midfrontal and superior temporal regions. The loss of cortical thickness in the inferior parietal region is accompanied by diminished muscarinic receptor binding, but not by significant reductions in neuron populations. This absence of neuronal loss, coupled with cortical thinning and reductions in muscarinic binding sites, implies a shrinkage or disappearance of the constituent dendrites of the neuropil.

Despite numerous investigations, changes in muscarinic cholinergic receptors in Alzheimer neocortex are similarly unsettled. This topic has been well reviewed recently[29]; in brief, some studies have found

no alterations in muscarinic cholinergic receptor density in AD,[28,30] while others describe significant reductions.[25] This situation is further complicated by dividing muscarinic receptors into subtypes,[31] the functional significance and anatomic (presynaptic or postsynaptic) localizations of which are not yet totally clear. We found significantly lower levels of muscarinic receptors in Alzheimer midfrontal and superior temporal cortex when compared with controls, using methylscopolamine as ligand.

Summarizing the results of this study, we note that PD is characterized morphometrically by major losses of medium and large neurons in the midfrontal and superior temporal regions, with resultant cortical narrowing. Losses of lesser magnitude occur among small neurons. The inferior parietal cortex is also thinned; but because neuron counts are undiminished and there is an increase in neuron density, this probably reflects loss of neuropil, rather than perikarya. No correlations were seen between the degree of cortical atrophy and age of onset, disease duration, or counts of Pick bodies. It may be significant, however, that in all 5 cases analyzed, disease duration exceeded, and in 4 of the cases, doubled or tripled, that reported in the literature as the average for PD.[32] Therefore, a ceiling effect may be operative. The neurochemical data imply maintenance of cortical cholinergic input from comparatively intact large neuron populations in the substantia innominata. There is, however, loss of intrinsic cholinoceptive neurons in the neocortex of the frontal and temporal lobes and of muscarinic receptor sites presumably located in the neuropil of the inferior parietal lobe. PD resembles AD, but is of greater severity as regards neuron loss and atrophy in the midfrontal and superior temporal regions, while the parietal cortex is more affected in AD. We find no evidence that PD and AD represent points along a common spectrum. Cholinergic input from the basal forebrain is diminished in AD (unlike PD), but intrinsic muscarinic receptors are reduced in both conditions as studied with N-methyl scopolamine as ligand.

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