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Distribution of neurofibrillary tangles and senile plaques in the cerebral cortex in postencephalitic parkinsonism

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Postencephalitic parkinsonism is characterized neuropathologically by severe loss of pigmented neurons in the substantia nigra and the presence of high densities of neurofibrillary tangles in several brainstem structures. In 5 cases of postencephalitic parkinsonism, we observed that the neurofibrillary tangle distribution in the cerebral cortex predominated in the hippocampus and entorhinal cortex. In the prefrontal and inferior temporal cortex, neurofibrillary tangles were preferentially localized in layers II and III. This pattern contrasts with the neurofibrillary tangle distribution observed in neocortical areas of Alzheimer's disease cases, where neurofibrillary tangles are denser in layer V than in layer III. These results suggest that specific elements of the cortical circuitry might be differentially affected in postencephalitic parkinsonism as compared to Alzheimer's disease, and that cortical involvement is likely to be a common feature of this condition.

Neurofibrillary tangles (NFT) and senile plaques (SP) are observed in different neurodegenerative disorders including Alzheimer's disease (AD), Down's syndrome, dementia pugilistica, progressive supranuclear palsy, and amyotrophic lateral sclerosis/parkinsonism-dementia complex of Guam. In the cerebral cortex of AD cases, NFT and SP are preferentially located in the hippocampal formation and neocortical association areas, whereas primary motor and primary sensory areas are less affected [1, 12, 13, 16, 20, 23, 26]. Neocortical NFT densities have been correlated with the loss of specific populations of pyramidal cells, suggesting that neuronal subsets could be more vulnerable, whereas other neuronal populations are relatively resistant to the degenerative process [13, 16]. The laminar and regional NFT and SP distribution as well as the patterns of neuron loss suggest that a selective disruption of specific elements of the cortical projection systems occurs in AD, leading to a global neocortical isolation syndrome that is clinically reflected as dementia [12–17, 20, 23, 26]. Neurofibrillary tangles are also observed in several subcortical structures in the brain of patients who had been exposed to the pandemic of influenza in the period 1915–1930 and who later developed postencephalitic parkinsonism (PEP), [6, 19–22, 27]. The brain areas where NFT were chiefly described include basal ganglia, nucleus basalis of Meynert, hypothalamus, substantia nigra, locus coeruleus, raphe, periaqueductal gray matter, and reticular formation [19–22, 27]. In addition, NFT have been detected in the Ammon's horn of the hippocampus [19]. However, such lesions have not been described so far in the neocortex of PEP patients. Dementia is not a common feature of PEP, and in most cases the clinical presentation is dominated by the extrapyramidal symptoms which almost invariably develop after the acute phase of the disease [6, 18, 25]. In this context, it is relevant to compare the distribution of cortical NFT and SP in PEP cases to that observed in AD, in order to assess whether the formation of such lesions constitutes a feature of PEP or is more likely to be related to aging or coexisting dementia.

The brains of 5 patients presenting with PEP (67.4 ± 5.9 year old, age range: 59–75 years) were obtained at autopsy (postmortem delay 4–24 h) and fixed in 4% paraformaldehyde for 24 h, and then stored in a 10% formalin solution. The clinical and neuropathological

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data were obtained from the clinical records of the Department of Psychiatry, University of Geneva School of Medicine, Geneva, Switzerland, and the Division of Neuropathology, Mount Sinai School of Medicine, New York. The patients were born between 1892 and 1912 and all had been affected by the pandemic of influenza in the years 1918–1924. The clinical presentation of all the cases was consistent with the diagnosis of PEP and included episodes of oculogyric crises, somnolence, inversion of the day/night rhythm, mutism, and development of a parkinsonian symptomatology [6, 18]. Neuropathological evaluation of the 5 cases revealed the typical features of PEP [7, 19, 21, 27] with loss of pigmented neurons in the substantia nigra, presence of NFT in the substantia nigra and several other brainstem nuclei, cell loss in the globus pallidus, and gliosis. Lewy bodies were not observed. Three of the patients (cases 1–3; 59, 65, 75 years old, respectively) had no cognitive impairment. Review of the medical records of case 4 (68-year-old) revealed a slight temporospatial and cognitive impairment. Case 5 (70-year-old) presented with dementia of the Alzheimer type.

Thirty μm-thick sections were cut on a cryostat and stained with modified thioflavine S [11, 28], Gallyas, or modified Globus techniques as previously described [12]. In addition, sections were processed with a specific antibody to the microtubule-associated protein tau. Characterization of this antibody has been fully reported elsewhere [4, 5, 8]. Briefly, 30 μm-thick sections were incubated overnight with the anti-tau antibody at a dilution of 1:2,000. Following incubation, sections were processed by the avidin-biotin method using a Vectastain ABC kit (Vector Laboratories), and diaminobenzidine. Some sections stained with the anti-tau antibody were counterstained with hematoxylin-eosin to clarify the cytoarchitecture. The following areas were analyzed (numeration according to Brodmann's nomenclature): 9 and 46 in the prefrontal cortex, 20 and 21 in the temporal cortex, entorhinal cortex (area 28) and hippocampus proper. All the sections were systematically surveyed and lesions were counted using a computer-assisted image analysis system consisting of a Zeiss Axiophot photomicroscope equipped with a motorized stage, a high sensitivity SIT camera, a DEC 3100 workstation and Macintosh II microcomputer, and custom software. Quantitative analyses were performed on thioflavine S-stained sections. Comparable results were obtained on tissues stained with the antibody to tau protein [28]. On each slide, NFT were counted under five to ten 1 mm-wide traverses in layers II–III and V–VI separately in the areas 9 and 20, in layers II–III and IV–VI in the entorhinal cortex, and in the pyramidal layer of the hippocampus proper.

The analysis of NFT distribution in the cerebral cortex of the 5 PEP cases showed large differences in densities (Table I). In the pyramidal layer of the hippocampus, values ranging between 3 and 40 NFT per mm traverse were observed (Fig. 1A). In this region most NFT were observed in the CA1 field. Comparable NFT densities were found in the subiculum. The entorhinal cortex contained the highest NFT counts in the cerebral cortex, in particular in layers II and IV (Fig. 1B; Table I). Counts in layer II ranged from 5 to 30 per mm traverse, and 4 to 20 per mm traverse in layer IV. In the neocortical areas of the temporal lobe, NFT were predominantly located in layers II and III, whereas they were less numerous in layers V and VI (Fig. 1C, D; Table I). Depending on the case, there were 21–50% more NFT in the superficial layers as compared to the deep layers in area 20. Values were comprised between 2 and 39 per mm traverse in layers II and III, and 1 and 31 per mm traverse in layers V and VI. Very rarely, NFT were encountered in the prefrontal cortex with counts reaching up to 2 NFT per section. There were moderate SP densities in the cortical areas of these PEP cases. Senile plaques were observed in the Ammon's horn of the hippocampus with average values ranging between 1 and 8 per mm traverse. Comparable SP densities (2–12 per mm traverse) were found in the neocortex. Senile plaques were predominantly located in the supragranular layers of neocortical areas. One case (case 2) was devoid of SP, and case 5 displayed numerous SP in all cortical regions with densities as high as 30 SP per mm traverse in temporal and prefrontal neocortex. In addition, cases 4 and 5 displayed numerous tau-positive neuritic elements in the neuropil (Fig. 1C), and within SP in all the cortical areas investigated.

These results demonstrate that NFT and SP are not uncommon pathological features in the cerebral cortex of PEP cases, although their density is not as elevated as in AD cases. Moreover, the distribution of lesions, in

TABLE I

NEUROFIBRILLARY TANGLE COUNTS IN THE CEREBRAL CORTEX OF PEP CASES

Results represent means ± S.D. and are expressed as NFT counts/mm cortical traverses in the pyramidal layer of the hippocampus (CA1), entorhinal cortex (area 28), and inferior temporal cortex (area 20). Neocortical areas are numbered according to Brodmann's nomenclature. See text for details.

Area	Case 1	Case 2	Case 3	Case 4	Case 5
CA1	4.5±0.5	4.4±0.3	25.7±3.5	22.0±3.3	35.1±3.2
28 Layers II–III	6.5±1.0	20.8±3.5	25.0±2.4	26.7±1.2	28.0±0.6
Layers IV–VI	5.1±0.7	12.8±2.9	17.4±2.0	15.5±2.2	17.8±1.3
20 Layers II–III	3.0±0.3	5.6±1.6	34.1±3.9	10.9±1.1	10.1±2.9
Layers V–VI	1.5±0.2	3.1±1.5	27.3±3.0	5.8±2.6	4.7±1.3

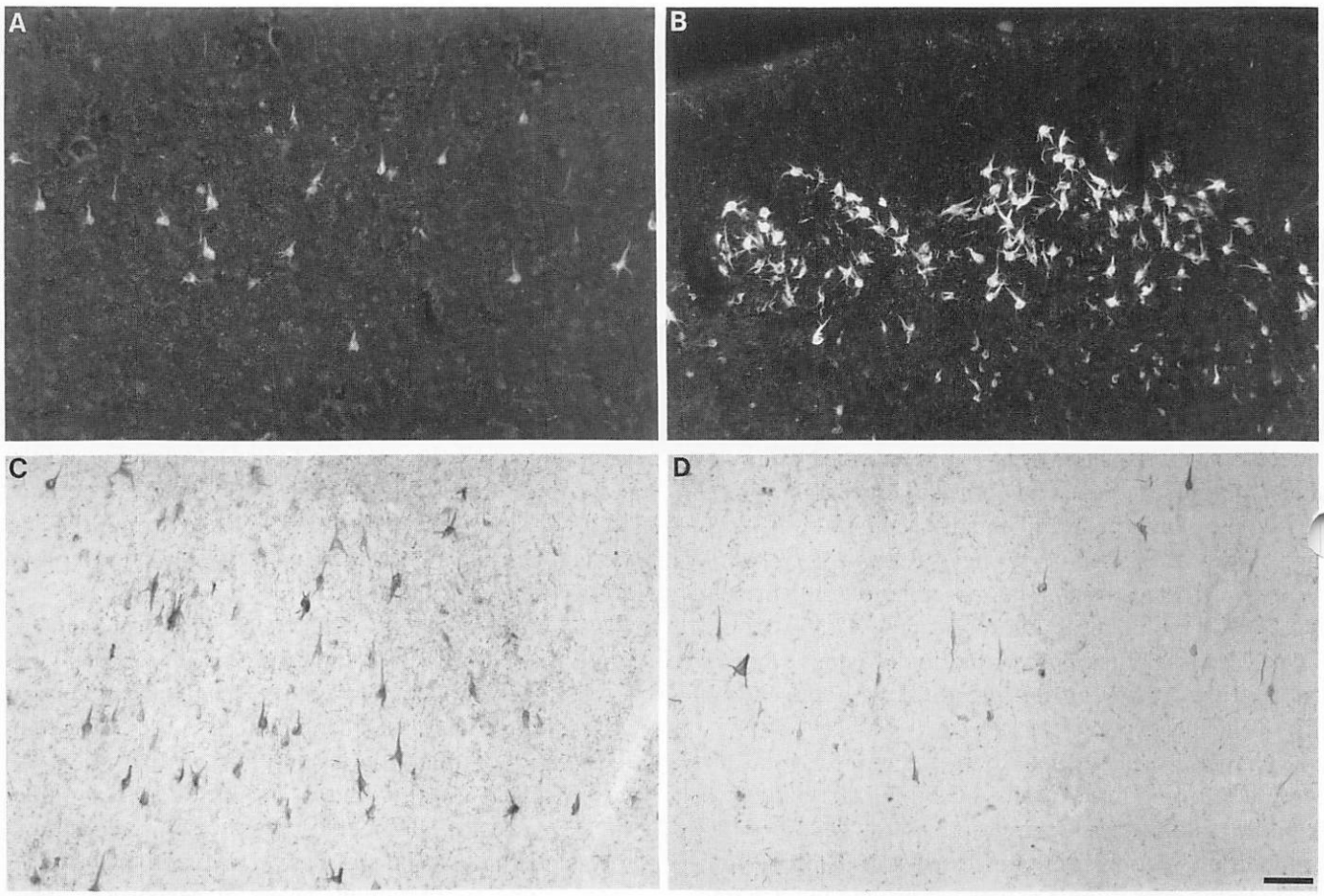

Fig. 1. Examples of typical alterations observed in the postencephalitic parkinsonism (PEP) brains. A: neurofibrillary tangles (NFT) in the CA1 field of the hippocampus. B: NFT in layer II of the entorhinal cortex. C, D: NFT in layer III (C) and V (D) of inferior temporal cortex (area 20); note the higher density in the superficial layers and the numerous tau-positive elements in the neuropil. Materials were stained with thioflavine S (A,B), or with the antibody to tau protein (C,D). Bar = 100 μm.

particular of NFT, in the cerebral cortex of PEP patients displays substantial differences with that observed in AD. For instance, the neocortical involvement is less pronounced in PEP than in AD cases, with most NFT located in the inferior temporal cortex and low NFT counts in the prefrontal cortex. Alzheimer's disease cases usually show abundant NFT and SP throughout the neocortex, with a preferential distribution in high level association areas of the frontal and temporal neocortex [1, 12, 13, 16, 20, 23, 26]. However, the hippocampal formation in both PEP and AD cases show comparable patterns of NFT and SP distribution. In addition, the presence of tau-immunoreactive NFT, as well as tau- positive elements in the neuropil and in SP suggests an abnormal deposition of this protein in the cerebral cortex of PEP cases. Abnormal tau protein species, referred to as tau 55, tau 64 and tau 69 [5, 8], are accumulated in neurons prone to NFT formation in AD and in Down's syndrome brains [5, 8, 9]. It is likely that in PEP cases NFT display a biochemical profile comparable to that observed in AD. This could be reflected in the fact that ultrastructurally NFT of PEP cases do not differ from those found in AD [19].

The laminar distribution of NFT in neocortical areas of PEP cases differed from that observed in AD. It has been reported that in AD, most NFT occur in layer V, whereas they are less numerous in layer III [12, 13, 16, 17, 20, 23]. It is worth noting that in all the PEP brains, NFT were more numerous in the supragranular than in the infragranular layers of the neocortex. In fact, the laminar NFT distribution in the neocortical areas of PEP cases is reminiscent of that observed in cases presenting with Guam amyotrophic lateral sclerosis/parkinsonism-dementia complex [17], dementia pugilistica [2], autism associated with severe self-injury behavior [15], and progressive supranuclear palsy [14]. In all these conditions, quantitative analyses have revealed that in prefrontal and inferior temporal cortex, NFT were preferentially located in layers II and III, whereas much lower values were observed in layers V and VI [2, 14, 15, 17]. It has been proposed that in these conditions, the neuronal populations within layers II and III that appear

to be selectively affected are spared to a certain degree in AD, however additional neuronal subsets in layers V and VI are dramatically affected in AD cases [17]. It is, however, not clear how the distribution of NFT is related to the clinical presentation of PEP. In this context, the severe involvement of subcortical structures seen in PEP brains is likely to play a key role in the progression of the disease [3, 6, 10, 18, 21, 27, 29], while dementia in AD might be more related to the massive cortical pathological changes. Nevertheless, it is worth noting that the same cortical areas that appear to be involved in the early stages of AD (i.e., hippocampus, entorhinal cortex and inferior temporal cortex [24]), also display NFT and SP formation in PEP cases. However, the relative paucity and differential laminar distribution of NFT in the neocortical areas of PEP cases as well as the highly variable amounts of amyloid deposition argue against co-occurrence of senile dementia in most of these patients (at least 3 out of 5 cases in the present series). In addition, recent studies have demonstrated that the progression of deterioration in PEP patients is not related to aging but is due to factors inherent to the disease itself [3, 10, 29]. It should be noted however that two of our cases presented with either early symptoms of possible AD (case 4), or with dementia of the Alzheimer type (case 5), and displayed higher SP densities in the cortical areas investigated. In these cases, the presence of AD superimposed onto pre-existing PEP cannot be ruled out, even though the laminar distribution of NFT in the neocortex clearly differed from that usually observed in AD [12, 13, 16, 17, 20, 23]. These results suggest that NFT formation in PEP may affect different neuron populations as compared to AD, and that severe cortical involvement in this disease is more common than previously thought.

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