TOPOGRAPHY OF PICK INCLUSION BODIES IN HIPPOCAMPAL NEURONES OF DEMENTED PATIENTS
A Quantitative Study
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ABSTRACT

Topographic analysis was performed on the distribution of argyrophilic inclusions in hippocampal neurones of patients with Pick’s dementia. A semiautomated scanning stage microscope linked potentiometrically to an XY pen recorder permitted the plotting of cytoarchitectonic “scattergrams” from the sequentially screened hippocampal formations. The density of Pick body-bearing nerve cells per cubic mm. of (pyramidal) cortex was quantified by measuring the area of each of six “zones” with a digitizer and programmable calculator.

The ranking orders of relative severity showed that neurones in Rose’s H1 field, the adjacent subiculum, and the entorhinal cortex are severely involved; that H2 and the presubiculum are less afflicted; and that the endplate is the least affected of all. The similarity of these predilections to those already demonstrated for Hirano bodies, granulovacuoles and neurofibrillary tangles in Alzheimer’s disease suggests that a specific neurotransmitter defect may also underlie the dementia of Pick’s disease.

INTRODUCTION

Although the spherical argyrophilic bodies found in the cytoplasm of hippocampal neurones in many cases of Pick’s disease (Fig. 1) have frequently been described both at light-microscopic (5, 7, 12, 19) and at electron-microscopic magnifications (18, 20), almost nothing has been reported about their precise topographic distribution within the pyramidal cell layer of the hippocampal cortex. Even in van Mansvelt’s treatise (15), perhaps the most comprehensive study of this disease since Alzheimer’s original description (1), no mention of regional predilection within the mesial temporal neurone population is made. One allusion of a topographic nature is found in the study by Constantinidis and colleagues (6) of ten cases of Pick’s disease, in which these argyrophilic inclusions were said to be ‘very abundant’ in the small neurones of the dentate fascia, in the pyramidal cells of the subiculum and in the Sommer sector; ‘never observed’ or ‘very rare’ in their ‘HD area’, corresponding to the presubiculum; and noted to ‘reappear at the HC area, that is, the limit of temporal neocortex’ or entorhinal region. From three autopsied cases, Malamud and Waggoner (14) felt that the presubiculum and the cornu Am-
monis were ‘resistant’ to the *atrophic* changes in Pick’s disease, whereas the rest of the hippocampus (Brodmann’s areas 34, 28 and 35) was ‘regularly affected’; but these authors did not mention the location of Pick bodies specifically. In another case Ferraro and Jervis (11) noted ‘a few’ argentophilic inclusions in the Sommer sector of the cornu Ammonis but nowhere else, ‘in spite of an extensive investigation’ using Bielschowsky and von Braunmühl stains. None of the foregoing authors provided any detailed quantitative data of their impressions.

Because neurofibrillary tangles, granulovacuolar degeneration and Hirano bodies had been found to show a striking predilection for certain sectors of the hippocampal cortex in earlier quantitative studies in our laboratory on brains of patients with Alzheimer’s disease (2, 3), we attempted in the present investigation to determine if any similar topographic selectivity exists for the Pick bodies in a condition bearing many clinical and pathological similarities to the much more common organic dementia already studied.

**MATERIALS AND METHODS**

The brains of two demented patients were examined. The first (Case #1) was a carpenter who developed gradual deterioration of intellect commencing at approximately 54 years of age, accompanied by emotional lability, shortening attention span, marked personality changes, and ending in a profound dementia with disorientation to time, place and person. He died of bronchogenic carcinoma at age 64. The second (Case #2) was a machine operator who became forgetful and disinterested at about 67 years of age, eventually developing impaired judgement and insight, irrational speech, very poor recall and disorientation in all spheres. He was frequently dangerous to other patients, and would often be found eating feces. He died at age 74 of pneumonia and recurrent myocardial infarction.

At autopsy, both brains revealed striking (‘knife-edge’) atrophy of the frontal and especially the temporal lobes, but sparing the posterior 2/3 of each temporal gyrus. Fixed brain weights were 1200 grams and 985 grams, respectively.

Microscopically, neuronal loss and gliosis were noted in both cases in frontal and temporal cortex; a few senile (neuritic) plaques in the parietal cortex of, and a rare plaque in hippocampus of the second case only; mild granulovacuolar degeneration of hippocampal neurones in both; rod-like Hirano bodies in hippocampal cortex of both; and huge numbers of swollen neurones frequently bearing spherical amorphophilic cytoplasmic structures (Fig. 1a), displaying the argyrophilic staining typical of Pick “inclusion” bodies (Figs. 1b, c), in the hippocampus of both cases and also less frequently in the frontal, parietal and occipital cortex, thalamus, hypothalamicus and substantia nigra of the second patient. Neurofibrillary tangles of Alzheimer were never found in the first, and were only rarely noted in the pontine reticular substance of the second case. Electron-microscopic features of the Pick bodies (Figs. 1d, e) were typical of those already described in other cases of Pick’s dementia (18, 20), exhibiting large numbers of randomly arranged linear non-twisted neurofilaments averaging 100 Å in diameter with a triple-density longitudinal profile, situated within a non-membrane-limited spherical matrix of dense, osmiophilic aggregates.

After the brainstem and cerebellum had been removed from each formalin-fixed brain, the entire hippocampus (from a coronal level 2 cm. posterior to the rostral tip of the temporal lobe, to the plane of the callosal splenium) was excised, sliced sequentially in the coronal plane, and all the tissue blocks serially sectioned in paraffin at 6μ. The middle section from each such tissue block (2) was stained with hematoxylin and eosin and Luxol fast blue, and the very next serial section stained with the Bodian metallic silver impregnation technique. The area to be screened included the hippocampal formation proper (Ammon’s horn), the prosubiculum, the subiculum and presubiculum of the hippocampal gyrus, and the parahippocampal gyrus as far laterally as the collateral sulcus.
Fig. 1. A—Spherical amphophilic Pick bodies in cytoplasm of two hippocampal neurones. Hematoxylin and eosin stain, ×850. B and C—Argyrophilic Pick inclusion bodies. Bodian stain, ×890. D and E—Electron-micrographs of Pick body; formalin-fixed, post-fixed in gluteraldehyde and osmium tetroxide, stained with uranyl acetate and lead citrate, mags. ×900 and ×14,200. Note the eccentric location of the nucleus (D), and the tubular linear profiles (E).

Fig. 2. Topographic “scattergrams” showing location of fields containing Pick bodies within the hippocampus of Cases 1 (A) and 2 (C). Small “d” indicates fascia dentata. Original mag. ×108. The coronal microscopic sections from which these were prepared are shown in (B) and (D), respectively.
This complete area within the inked border outlined on each coverslip was then scanned sequentially with a Wild M501 semi-automated microscope at 400× magnification, with a square ocular (Weibel) graticule. With the gears of the mechanical stage potentiometrically coupled to an XY Pen Recorder (Rikadenki BW200), the recording pen produced a dot at the location of each visual field containing one or more neurones with an intracytoplasmic argyrophilic Pick body (Figs. 2). Such fields were considered “positive” whether or not the affected cells’ nucleus or nucleolus could also be visualized. Positive fields within the dentate fascia, however, were not plotted; its numerous granule cells also bearing Pick bodies were ignored in the present study. A total of 10,084 microscopic fields was examined, each representing 0.051 mm² in a section 5.85 μ in true thickness.

The outer borders of the hippocampus and the configuration of the fascia dentata were also drawn onto the ‘scattergrams’ with the pen-recorder by tracking these with the microscope (Figs. 2a, c). These demarcations permitted easy comparison with the histological sections from which each “scattergram” had been made (Figs. 2b, d).

In each case, three coronal sections of the left hippocampus were thus surveyed, commencing in the anteroposterior plane only when a well-formed capital “C” configuration of the dentate fascia was visible. (Data from right-sided hippocampi are not presented, since examination of an additional 1,685 fields of right hippocampal cortex showed no major differences from left-sided results.)

The overall area of hippocampal cortex scanned was subdivided into six “zones”—the end-plate (Rose’s fields H₃, H₁, and H₂); the H₄ field of Rose; the lateral portion of H₁ (the Sommer sector); the medial portion of H₁ (prosubiculum) and contiguous subiculum; the presubiculum and parasubiculum; and the entorhinal area, including the para-pyramidal gyrus (Fig. 3). Boundaries between these 6 “zones” were drawn onto the “scattergrams”, and the cortical area within each “zone” was measured (in square mm.) with a digitizer (Hewlett-Packard) linked to a calculator. Since the magnification from the actual glass slides was known (X108), as was the true thickness of the paraffin sections, it was possible to calculate the number of positive fields per cubic mm. of paraffin-embedded tissue (i.e., an “Adjusted Field Index”) . . . for each “zone”. The Indices of all the slides of each case were averaged to derive a Mean Adjusted Field Index for each “zone” (Tab. 1).
The relative density of positive fields can be considered a very reasonable reflection of the relative density of the actual number of Pick bodies, since (although positive fields contained anywhere from one to 15 inclusion-bearing neurones) the mean number of nerve cells with Pick bodies in any one positive field showed no meaningful variation, ranging only between 3.83 and 3.97 in slides of Case 1 and between 3.07 and 3.38 in slides of Case 2 (Tab. 2). Thus a rank order number could be assigned to each Mean Adjusted Field Index (see numbers in parentheses, Table 1), with "1" representing the largest and "6" the smallest Index of each case.

RESULTS

For the first patient, the ranking order (in decreasing severity) of hippocampal Pick body formation was: \( H_1 > \) entorhinal \( > \) subiculum \( > \) \( H_2 > \) presubiculum \( > \) endplate. In the second case, the rank order was: entorhinal \( > \) subiculum \( > \) \( H_1 > \) presubiculum \( > \) \( H_2 > \) endplate.

Although these rank orders differ somewhat between the two cases, the same general tendencies are very apparent: neurones of Rose’s \( H_1 \) and adjacent subiculum are severely affected by Pick body formation, as are those in the entorhinal cortex (Tab. 1). By contrast, the \( H_2 \) zone of Rose and the presubiculum are much less involved, and the end-plate is the least affected of all (Tab. 1).

While the “raw” Pick body counts have been corrected only for cortical volume surveyed and not for neuronal population densities, it is highly unlikely that the marked regional selectivity observed in this study can be attributed merely to possible differences in nerve cell numbers in the hippocampal “zones”; for example, the \( H_2 \) field of Rose, much less affected by Pick body formation, has been shown to contain the highest density of neurones in the entire hippocampal formation (8).

A much more likely explanation is that a chemical specificity of particular “chains” of neurones linked by some common neurotransmitter underlies these regional predilections. Evidence for a specific biochemical defect in Pick’s disease is not yet available; but in Alzheimer’s disease strength is growing for

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<td>MEAN ADJUSTED FIELD INDEX: Mean Number of Pick Bodies per cu. mm. of Cortex</td>
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the hypothesis that the observed cortical deficiency of choline acetyltransferase (4, 9, 16, 17) reflects a particular jeopardy of the cholinergic system, upon which the integrity of the memory circuitry of the limbic apparatus may depend (10). It is therefore of special interest that in hippocampi of patients with Alzheimer's dementia we have already found very similar ranking orders of predilection for the rod-like Hirano bodies, for granulovacuolar degeneration of Simchowicz, and especially for neurofibrillary tangles of Alzheimer (2, 3). For all of these histological changes, once again H₁ (Sommer sector), the subiculum and the entorhinal region are heavily involved, whereas the "resistant" H₂ field, the endplate and especially the presubiculum are relatively spared.

Because Pick's and Alzheimer's disease have much in common, not only clinically but also pathologically, sometimes even occurring within the same brain (13), the topographic evidence in the patients reported here suggests that search for a similar neurotransmitter defect in the dementia of Pick's disease might prove fruitful.

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