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MODULAR AND LAMINAR PATHOLOGY OF BRODMANN'S AREA 37 IN ALZHEIMER'S DISEASE

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Abstract—Previous studies suggested a relationship between severity of symptoms and the degree of neurofibrillary tangles (NFTs) clustering in different areas of the cortex in Alzheimer's disease (AD). The posterior inferior temporal cortex or Brodmann's area (BA 37) is involved in object naming and recognition memory. But the cellular architecture and connectivity and the NFT pathology of this cortex in AD received inadequate attention. In this report, we describe the laminar distribution and topography of NFT pathology of BA 37 in brains of AD patients by using Thionin staining for Nissl substance, Thioflavin-S staining for NFTs, and phosphorylated tau (AT8) immunohistochemistry. NFTs mostly occurred in cortical layers II, III, V and VI in the area 37 of AD brain. Moreover, NFTs appeared like a patch or in cluster pattern along the cortical layers III and V and within the columns of pyramidal cell layers. The abnormal, intensely labeled AT8 immunoreactive cells were clustered mainly in layers III and V. Based on previously published clinical correlations between cognitive abnormalities in AD and the patterns of laminar distributed NFT cluster pathology in other areas of the brain, we conclude that a similar NFT pathology that severely affected BA 37, may indicate disruption of some forms of naming and object recognition–related circuits in human AD.

Key words: neurofibrillary tangles, posterior inferior temporal cortex, Brodmann's area 37, phosphorylated tau, laminar distribution, memory impairment.

Neurons in the cerebral cortex are organized horizontally into lamina and vertically into columns and modules. These organized neurons, with similar response properties, form fundamental anatomical and physiological units of the cortex (Fujita et al., 1992; Saleem et al., 1993; Tanigawa et al., 1998).

In Alzheimer's disease (AD), the characteristic neuropathological lesions of neurofibrillary tangles (NFTs) exhibit a specific laminar and regional distribution in different areas of brain (Hof and Morrison, 1990; Hof et al., 1992; Hof, 1997). The modular/columnar organized NFT pathology of the hippocampus, the entorhinal cortex and surrounding limbic structures in AD has been described in the literature (Van Hoesen and Solodkin, 1993; Van Hoesen et al., 2000). The NFTs pathology in hippocampus specifically affect the regional cells of origin of afferent and efferent neuronal fibers (Hyman et al., 1984; Van Hoesen et al., 1991). Prominent loss of pyramidal neurons in layers III, V and VI in visual and auditory association cortices also occurs in AD (Hof and Morrison, 1990; Van Hoesen and Solodkin, 1993; Joyce et al., 1998). This multi-layered NFTs pathology may be due to the affected feed forward and feedback corticocortical axons arising from layers II–VI of the cortex (Van Hoesen, 1995).

Previous studies have shown that the AD-related disorders are strongly related to the NFT formation in cortical areas of multimodal association and the NFTs in inferior temporal cortical areas signals more severe memory impairment and impending signs of dementia (Hof et al., 1990; Hof 1997). The positron emission tomography and electrophysiological measurements have designated posterior inferior temporal cortex, mapped as Brodmann's area 37 (BA 37), for object naming and visual object recognition memory (Tanaka, 1997; Harasty et al., 1999; Haxby et al., 2001 and Nakamura et al., 2000). But the NFT neuropathology and neuronal connectivity studies of BA 37 received inadequate attention in the literature. In the present investigation, we focused on BA 37 considering that progression of NFT neuropathology in this cortical area may have neurobehavioral consequences in AD patients.

EXPERIMENTAL PROCEDURES

Human brain samples

AD brains were obtained from 10 individuals at autopsy (University of Iowa Deeded Body Program, Iowa City, IA, USA) with duration of dementia from 3 to 15 years (AD cases are summarized in Table 1) and age-matched control brains were obtained at routine autopsy, from patients dying without any history of neurological or psychiatric illness.

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Abbreviations: AD, Alzheimer's disease; AT8, abnormally phosphorylated tau; BA 37, Brodmann's area 37; FB, Fast Blue; NFT, neurofibrillary tangles; PBS, phosphate buffer saline.

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Table 1. AD case demographics

Case	Age (years)	Sex	Duration of illness (years)	Brain weight (g)
A559	79	F	11	956
A569	89	M	10+	1630
A584	79	F	11	860
A596	82	M	12	1180
A599	85	M	5	1050
A630	83	M	5	986
A631	61	M	6+	1265
A660	82	M	4	1224
A666	78	M	12	1060
A669	88	F	3	950

Gross anatomical and neuropathological examination

The area of posterior inferior temporal cortex is routinely called BA 37. According to Brodmann's areal map of the human cortex, this posterior temporal cortical area is located lateral to the parahippocampal gyrus and the collateral sulcus; it occupies a position immediately adjacent to the posterior parahippocampal gyrus. The borders of this area are not clearly demarcated by functional or anatomical landmarks. The neuropathological lesions were consistent with AD diagnostic criteria. Nissl-staining was performed to reveal an altered laminar arrangement of neurons and to analyze the neuronal loss and gliosis in BA 37 by visual inspection. Cytoarchitectural comparisons with normal control Nissl-stained series were routinely performed.

Brain tissue preparation

All rapid autopsy brains from AD patients were collected, the temporal lobe was divided in blocks and immersion-fixed in ice-cold 4% paraformaldehyde solution in 0.1 M phosphate buffer saline (PBS) for 24–48 h. Fixed tissue blocks containing the hippocampus and adjacent temporal cortices were cut into 50-μm-thick frozen sections and stored frozen until processed. The thick frozen sections were further cut into series of thin, 5 μm, sections on freezing sliding microtome and stored in cryostorage solution until stained with Thionin for cytoarchitectonic delimitation.

Thioflavin-S histochemistry

Another parallel series of sections were stained with 1-% Thioflavin-S (Sigma, St. Louis, MO, USA) to survey AD pathology and to identify the laminar localization pattern, density of NFTs, and amyloid plaques in BA 37 of AD. The sections were pretreated in a 1:1 mixture of chloroform ($CHCl_3$)/absolute ethanol (EtOH) for 10 min and 95% EtOH and 70% EtOH for 10 min then quickly rinsed in water, and incubated in 0.1% Thioflavin-S for 5 min at room temperature in the dark. Finally, the sections were briefly differentiated in 80% EtOH solution and rinsed in water, and mounted with Aquamount. Sections were examined under a Leitz Diaplan fluorescent microscope using a 10× objective. The microscope was equipped with a camera and image analysis software, Neurolucida (MicroBrightField Inc., Colhester, VT, USA), to chart laminar localization and distribution patterns of NFTs in Thioflavin-S stained sections.

Immunohistochemistry

A series of free-floating sections from the posterior temporal cortex of each brain was processed for abnormally phosphorylated tau (AT8) immunohistochemistry using the avidin–biotin–peroxidase complex (ABC) method as described by Lee et al., 2004. Briefly, the sections were quenched with 0.1% H_2O_2 in 0.1 M PBS containing 0.4% Triton X-100 for 20–30 min. After washing in 0.1 M PBS, the sections were blocked with 5% normal goat serum containing 0.4% Triton X-100 in 0.1 M PBS for 1 h at room temperature followed by overnight incubation with primary antibody AT8 (1:1000; Innogenetics, Gent, Belgium) at 4 °C. After washing, sections were incubated sequentially with biotinylated goat anti-mouse secondary antibody (1:500 dilution; Vector Laboratories, Burlingame, CA, USA), an avidin–biotin–peroxidase complex (ABC Elite kit; Vector Laboratories, diluted 1:200 in 0.1 M PBS containing 0.4% Triton X-100), 0.03% 3,3′-diaminobenzidine (DAB) containing 0.25% nickel ammonium sulfate, and 0.01% H_2O_2 in 0.1 M PBS for 5–10 min. The sections were washed after each incubation steps. To detect any non-specific labeling, the negative control sections were identically incubated without the primary antibody.

Monkey surgery and Fast Blue (FB) dye injection

Two monkeys were used in this study. All surgical and experimental procedures used were approved by the Institutional Animal Care and Use Committee at the University of Iowa and conformed to the Society for Neuroscience guidelines on the ethical use of animals. Every effort was made to minimize the number of animals used and their suffering. The surgery and histological procedures performed were as described by Morecraft and Van Hoesen, 1998). Briefly, the retrograde tracer, 3–4% of FB dye (4 μl), was injected into the middle temporal gyrus in brain of monkey anesthetized with Nembutal. Following a 14 days of survival period, monkeys were anesthetized and perfused with 0.9% saline in 0.1 M cacodylate buffer and then a solution of 6% paraformaldehyde in the same buffer. The brains were dissected and post-fixed for 4–6 days at 4 °C. Prior to sectioning, the brains were stored overnight in a solution of 30% sucrose in phosphate buffer solution. The 50 μm thick sections of the brain were collected in series and mounted immediately on glass slides. These series of sections were stained for Nissl substance for cytoarchitectural analysis and for fluorescent data analysis.

RESULTS

Thioflavin-S staining and cluster analysis

Under microscope, the NFTs were intensively labeled with Thioflavin-S containing histochemical stain. In AD brains, substantial numbers of NFTs accumulated throughout the posterior temporal lobe, but a high density of NFTs was registered in the entorhinal and perirhinal cortices of the parahippocampal gyrus (Fig. 1C and D) and both, intracellular and end-stage NFTs or extracellular tangles were also observed in this area. In area 37 of AD brain, NFTs formation was prominently distributed in infra-cortical layers II and III and in supra-cortical layers V and VI. Moreover, the NFTs appeared aggregated and were distributed along the cortical layers III, V and VI arranged in clusters or in patch-like patterns. The same cortical layers were also sparsely populated with cells. The modular and cluster pattern of NFTs prominently seen in layers V and VI of area 37 in an AD brain is illustrated in Fig. 1A and 1B. The NFT cluster size was also measured (Table 2).

Neurolucida charting

The Neurolucida charting of a cross-section through the inferior temporal cortex in an AD case depicting the topography and laminar distribution of NFTs is presented in Fig. 2. The figure shows a moderate NFT distribution in BA 37