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Re-examination of ex-boxers' brains using immunohistochemistry with antibodies to amyloid β-protein and tau protein*

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Summary. A histopathological study was carried out on the brains of eight ex-boxers (ages 56 to 83) using conventional histological staining methods and immunocytochemistry with antibodies to amyloid β-protein and the PHF-related tau protein. All cases showed a large number of tau-immunoreactive neurofibrillary tangles and also β-protein immunoreactive senile plaques in the cortex. In the areas with many neurofibrillary tangles, neuropil threads with tau-immunoreactivity were also observed, and some of the senile plaque lesions were surrounded by abnormal neurites with tau-immunoreactivity. Moreover, three cases revealed β-protein-type cerebrovascular amyloid deposits on both leptomeningeal and cortical blood vessels. The present observations indicate that the cerebral pathology of dementia pugilistica is very similar to that of Alzheimer's disease and suggest that these two disorders share some common etiological and pathogenic mechanisms.

Key words: Dementia pugilistica – Alzheimer's disease – Amyloid angiopathy – β-protein – Tau protein

Some ex-boxers who have experienced repeated head trauma show a degenerative brain disorder including psychosis, a progressive dementia and Parkinsonism, which is known as dementia pugilistica (DP) or "punch drunk" syndrome [25, 31]. A characteristic histopathological finding of the brain in this syndrome is the diffuse presence of neurofibrillary tangles (NFTs) throughout the cerebral cortex (especially medial temporal lobe) and the brain stem [11]. These NFTs are morphologically and immunohistochemically indistinguishable from those observed in the brain in Alzheimer's disease (AD) [14, 27, 36, 46]. Furthermore, recent immunocytochemical studies have revealed valuable new findings on brains with this disorder: many β-protein-immunoreactive senile plaque-like lesions [37] and significant numbers of NFTs with β-protein immunoreactivity [2] have been described in some cases with DP, suggesting etiological and pathological similarities between DP and AD.

In the present study we have re-examined a number of ex-boxers' brains using recently developed histochemical and immunocytochemical staining methods and report previously unknown findings on the cerebral pathology in this disorder.

Materials and methods

Formalin-fixed brain tissues from the temporal lobe were obtained from seven of the ex-boxers' brains originally reported by Corsellis et al. [11], and one additional case (Table 1); each case had been previously investigated to establish details of the boxer's career, and had undergone extensive neuropathological examination at Runwell Hospital, UK. Paraffin-embedded sections were stained by hematoxylin-eosin, alkaline Congo red, modified Bielschowsky's silver impregnation [50], and by immunocytochemical staining methods. Immunoperoxidase staining was carried out using the avidin-biotin peroxidase technique described previously [22]. The primary reagents were monoclonal antibody 4D12/2/6 raised against a synthetic peptide consisting of residues 8–17 of β-protein [1], an affinity-purified rabbit antiserum to the amino-terminal octapeptide of human cystatin C (AG8206) [30], and anti-tau antiserum [21]. The sections were immunostained with either 1:1000-diluted ascites fluid (4D12/2/6) or 1:500-diluted antisera (anti-cystatin C antiserum and anti-tau antiserum), and some sections were pretreated with 98% formic acid to enhance the immunoreactivity of amyloid-related proteins [28]. The immunospecificity of the staining produced by all three of these antibodies has been well characterized [1, 21, 28]. The lesions seen in all Congo red, silver impregnation and immunostained sections in each case were semiquantitatively estimated.

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Table 1. Semiquantitative estimation of neurofibrillary changes and β-protein deposits in eight cases with dementia pugilistica

Case	Age/sex (years)	Congo-red			Silver impregnation		Immunostain				
		PL	NFT	CAA	PL	NFT	ß-protein			Tau	
							PL	NFT	CAA	NFT	NT
1	56/M	++	++	++	+++	++	+++	–	++	++	++
2	61/M	++	+	++	+++	+	+++	–	++	–	–
3	61/M	–	+++	–	–	+++	+	+	–	+++	++
4	63/M	++	++	–	++	+	++	+	–	+	+
5	71/M	–	+++	–	++	+++	++	+	–	++	+
6	75/M	++	++	++	+++	++	+++	+	++	++	++
7	77/M	–	+++	–	–	+++	+	++	–	+++	++
8	83/M	++	+++	–	++	+++	++	+	–	+++	+

PL, senile plaque; NFT, neurofibrillary tangle; CAA, cerebral amyloid angiopathy; NT, neuropil thread; M, male
The following four grades of severity was observed under low power magnification: –, not observed; +, a few lesions were seen, but only after extensive search; ++, a substantial number of lesions were observed, but some optic fields were still free of lesions; +++, many lesions were visible in every field

Results

The results of Congo red, silver impregnation and immunohistochemical stainings are summarized in Table 1. Numerous Congophilic and argyrophilic NFTs that were distributed widely throughout the cortex examined were seen in all eight cases (Fig. 1A, C). These lesions were invariably immunoreactive to anti-tau antiserum (Fig. 1A, B), and some NFT-bearing neurons showed several tortuous apical and basal dendrites with tau

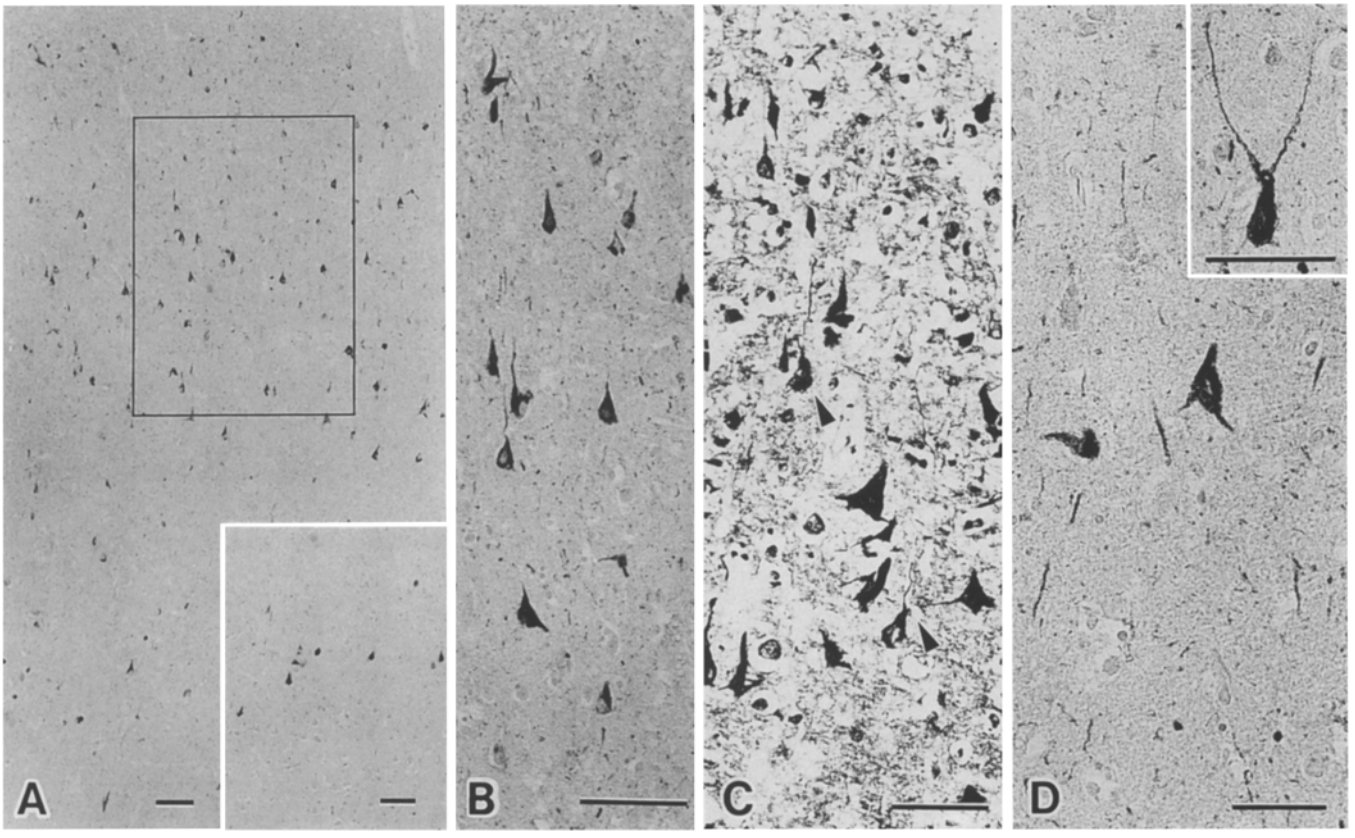

Fig. 1A–D. Representative pictures of neurofibrillary tangles (NFTs) and their related lesions seen in case 7. **A** Entire temporal cortex showing a large number of tau-immunoreactive NFTs. *Insert* reveals a few NFTs with β-protein immunoreactivity seen in the framed area. **B** Magnified picture of the NFTs intensely immunostained by anti-tau-antiserum. **C** Some NFT-bearing neurons (*arrowheads*) showing argyrophilic neuronal processes that are identified as dendrites. **D** In addition to nerve cells with strong tau-immunoreactivity, there are some short fibers (neuropil threads) with the same immunohistochemical reactivity. *Insert* is an immunolabelled neuron with tau-immunoreactive tortuous apical dendrites. **A, B, D** Tau immunostaining, **C** Silver impregnation. Bars **A–C** = 100; **D** = 50 μm

immunoreactivity (Fig. 1D, insert). Additionally, in areas with many NFTs there were substantial numbers of tau-immunoreactive abnormal neurites closely resembling the structures called "neuropil threads" which have been described in brains with AD [3, 4]; however, these pathological neurites were not greatly kinked or curled and appeared shorter than those reported in AD (Fig. 1D).

These eight cases also revealed variable numbers of morphologically diverse senile plaques in an extensive area of cortex (Fig. 2A, C), and three of them showed cerebrovascular amyloid deposits in both leptomeningeal and cortical blood vessels (Fig. 2A). Small vessels were heavily laden with amyloid deposits along the entire walls, which were frequently thickened, while amyloid deposits on the walls of large vessels were confined to the media or adventitia. The vessels in the subarachnoid space were more frequently involved than those in the cortex (Fig. 2B). All these cerebrovascular and senile-plaque amyloid deposits seen in the present

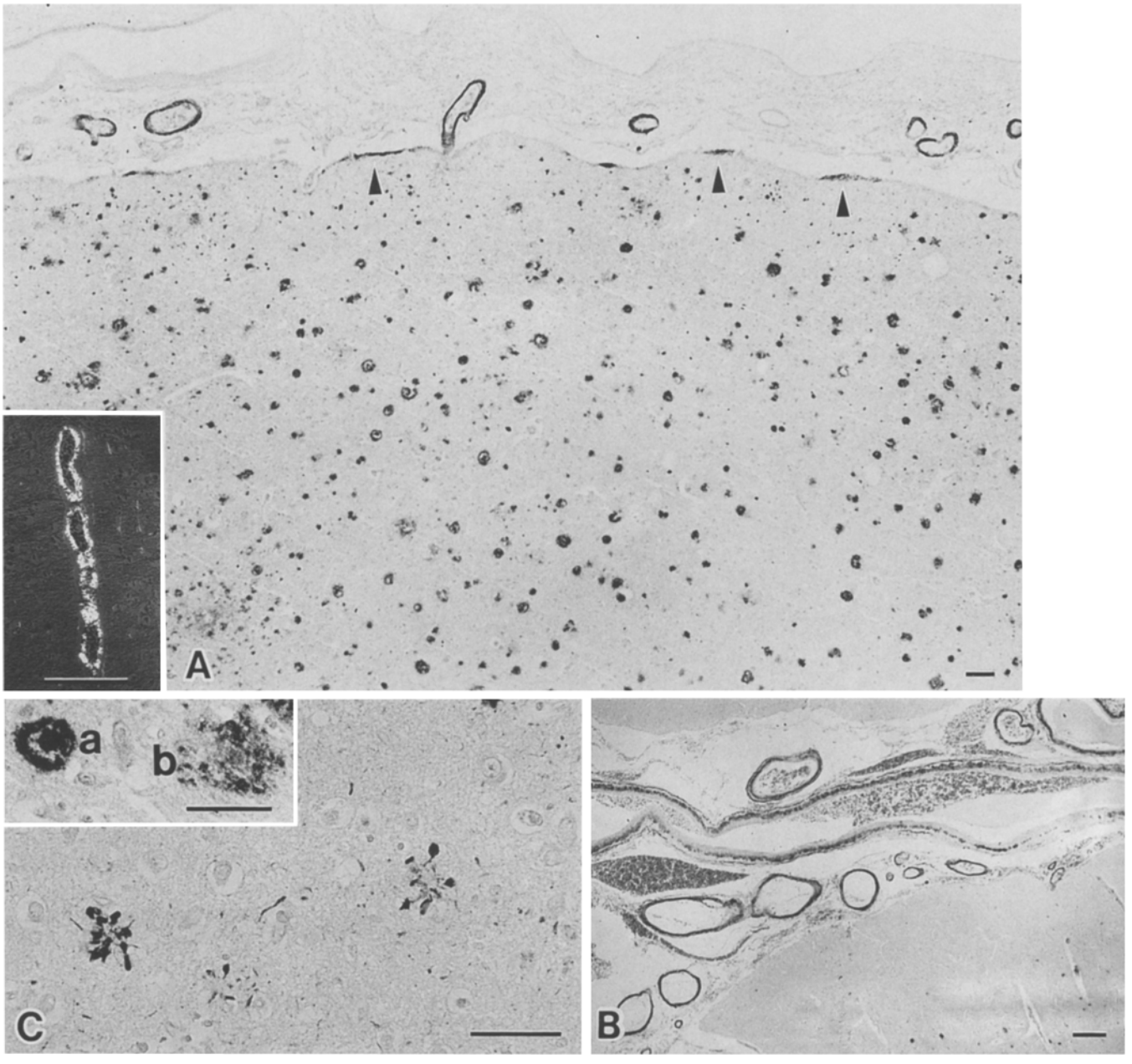

Fig. 2A–C. Representative pictures of cerebrovascular and senile plaque amyloid deposits. **A** and **B** are β-protein immunostaining. **A** Immunostaining with formic acid pretreatment shows cerebrovascular amyloidosis and various forms of β-protein-immunoreactive plaques (including early plaques and mature plaques) in case 1. *Arrowheads* show subpial band-like lesions with β-protein immunoreactivity and *insert* is a polarized view of cortical amyloid angiopathy after congo red staining. **B** Severe β-protein-immunoreactive amyloid deposition on the leptomeningeal vessels seen in case 2. Note the different patterns of vascular wall amyloid deposition between large and small vessels. **C** Tau immunostaining reveals dystrophic neurites in the periphery of senile plaques. *Insert* is β-protein immunostaining showing a typical mature plaque with a central amyloid core (*a*) and an early (diffuse) plaque (*b*). Bars **A, C** = 100; **B** = 200 μm

cases were consistently immunoreactive to anti-β-protein antibody (Fig. 2A, B, and insert of C) but were not stained by anti-cystatin C antiserum (data not shown). We could not find any close relationship between senile plaques and the vessels with amyloid angiopathy. However, three cases with cerebrovascular amyloidosis appeared to have a greater number of senile plaques with β-protein immunoreactivity than that seen in the remaining cases without cerebral amyloid angiopathy. In addition, degenerating neurites associated with some senile plaques were stained by anti-tau antiserum (Fig. 2C), and a small number of NFTs with β-protein immunoreactivity (the vast majority of them appeared to be extracellular tangles) were observed in six of these eight cases (Fig. 1A, insert). The detailed findings of β-protein-immunoreactive NFTs seen in some of the present cases have been reported by other investigators [2] and the topographical relationship between β-protein and tau-protein epitopes in NFTs has recently been described in AD [40, 49].

Discussion

It is well known that DP seen in some retired boxers who have experienced many fights might be caused by repeated head trauma [12, 25, 31], and early pathological studies of the brain in this disorder emphasized the presence of a large number of NFTs with the relative absence of senile plaques [11]. However, some recent immunohistochemical studies have revealed important new information on the cerebral pathology of DP. Abundant cerebral β-protein deposits resembling early [23] or diffuse [47] senile plaques [37] together with β-protein-immunoreactive extracellular NFTs [2] are common in this disease but there is no mention of neuropil threads or cerebrovascular amyloidosis in these reports.

NFTs and related lesions

The present study showed that the morphology and immunohistochemical properties of NFTs in DP are very similar to those reported in AD [14, 27, 36, 46]. Additionally, previously undescribed tau-immunoreactive abnormal structures were also observed in DP brains; these included neuropil threads and degenerating neurites located in the periphery of classical plaques. The former lesions (sometimes termed "curly fibers") have been described previously in AD [3, 4, 21, 29, 34, 48] where their distribution and number were found to correlate with those of NFTs [29, 35, 42]. However, in the present DP cases with many NFTs the degree of formation of neuropil threads seemed to be less prominent than that reported in AD cases [21, 29, 48], and any constant morphological relationship between neuropil threads and senile plaques was not observed. Accordingly, it seems likely that the pathological events involved in the formation of neuropil threads and the neuritic component of senile plaques might be different, although both lesions are composed mainly of bundles of the paired helical filaments containing tau-protein as a major antigenic component.

Cerebral amyloid angiopathy

Cerebral amyloid aniopathy (CAA) is observed in a variety of brain degenerative diseases [5], of which β-protein-type CAA [17] is the most common form seen in aged individuals including patients with AD [5]. In the present DP brains with a large number of NFTs and senile plaques, β-protein-type CAA was demonstrated in three of eight cases. It is well established that substantial numbers of typical senile plaques and/or CAA appear in the brains of aged non-demented individuals [13, 15, 43–45], and these age-related brain amyloids are also composed of β-protein [8, 9, 24]. However, two of the present three cases with CAA were aged 56 and 61, respectively, and both CAA and senile plaques rarely occur in these age-matched controls [15, 43–45]. It is concluded, therefore, that cerebrovascular amyloidosis with the abundant and diffuse presence of senile plaques seen in these three cases is not an incidental finding but a pathological condition associated with DP.

Pathological similarities in DP and AD brains

The present study clearly demonstrated that an extensive cortical involvement with neurofibrillary changes (NFTs, neuropil threads and degenerating neurites) and β-protein deposits (senile plaques and CAA) is a common pathological change in DP brains. In addition, all of the morphological and immunocytochemical findings described above indicate the many similarities in pathology between DP and AD. The detailed pathological mechanisms leading to the formation of NFTs, senile plaques and CAA in AD brains remain unclear. However, in DP patients with a long fighting career, repeated violent forces on the brain are considered to be the most important pathogenic factor that results in a demented state with Alzheimer's pathology; neurons injured by mechanical shearing of axons and/or dendrites develop NFTs with neuropil threads [41]. Similarly, β-protein precursors [26] in serum might leak into the brain parenchyma through a damaged blood-brain barrier [18]. Alternatively, on the basis of the idea of a neuronal derivation of β-protein [32], injured neurons could be potential sources for β-protein precursors.

It is uncertain whether or not head trauma is a direct causative factor in most cases of AD. However, some epidemiological studies [19, 33] have showed that there is a significantly greater incidence of head injury in patients with AD than controls, and a few cases [6, 7, 10, 20, 39] who insidiously developed AD after a single head trauma have also been reported, suggesting that head injury is one of the predisposing factors in development of AD. Although several etiological theories have been proposed for the pathobiology of AD [16], the present

study in conjunction with previous reports [2, 7, 37, 38] demonstrates that investigations of DP with a pathological resemblance to AD can provide useful information in considering the pathogenesis of Alzheimer-type dementia.

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References

1. Allsop D, Landon M, Kidd M, Lowe JS, Reynolds GP, Gardner A (1986) Monoclonal antibodies raised against a subsequence of senile plaque core protein react with plaque cores, plaque periphery and cerebrovascular amyloid in Alzheimer's disease. Neurosci Lett 68:252–256
2. Allsop D, Haga S, Bruton C, Ishii T, Roberts GW (1990) Neurofibrillary tangles in some cases of dementia pugilistica share antigens with amyloid β-protein of Alzheimer's disease. Am J Pathol 136:255–260
3. Braak H, Braak E (1988) Neuropil threads occur in dendrites of tangle-bearing nerve cells. Neuropathol Appl Neurobiol 14:39–44
4. Braak H, Braak E, Grundke-Iqbal I, Iqbal K (1986) Occurrence of neuropil threads in the senile human brains and in Alzheimer's disease: a third localization of paired helical filaments outside of neurofibrillary tangles and neuritic plaques. Neurosci Lett 65:351–355
5. Castaño EM, Frangione B (1988) Human amyloidosis, Alzheimer's disease and related disorders. Lab Invest 58:122–132
6. Claude H, Cuel J (1939) Démence pré-sénile post-traumatique après fracture du crane: cosidérations médico-légales. Ann Med Leg 19:173–184
7. Clinton J, Ambler MW, Roberts GW (1991) Post traumatic Alzheimer's disease: preponderance of a single plaque type. Neuropathol Appl Neurobiol 17:69–74
8. Coria F, Castaño EM, Frangione B (1987) Brain amyloid in normal aging and cerebral amyloid angiopathy is antigenically related to Alzheimer's disease β-protein. Am J Pathol 129:422–428
9. Coria F, Prelli F, Castaño EM, Larrondo-Lillo M, Fernandez-Gonzalez J, van Duinen SG, Bots GTAM, Luyendijk W, Shelanski ML, Frangione B (1988) β-protein deposition: a pathogenetic link between Alzheimer's disease and cerebral amyloid angiopathies. Brain Res 463:187–191
10. Corsellis JAN, Brierley JB (1959) Observations on the pathology of insidious dementia following head injury. J Ment Sci 105:714–720
11. Corsellis JAN, Bruton CJ, Freeman-Browne D (1973) The aftermath of boxing. Psychol Med 3:270–303
12. Critchley M (1957) Medical aspects of boxing, particularly from a neurological standpoint. Br Med J 1:357–366
13. Crystal H, Dickson D, Fuld P, Masur D, Scott R, Mehler M, Masdeu J, Kawas C, Aronson M, Wolfson L (1988) Clinico-pathologic studies in dementia: nondemented subjects with pathologically confirmed Alzheimer's disease. Neurology 38:1682–1687
14. Dale GE, Leigh PN, Luthert P, Anderton BH, Roberts GW (1991) Neurofibrillary tangles in dementia pugilistica are ubiquitinated. J Neurol Neurosurg Psychiatry 54:116–118
15. Dayan AD (1970) Quantitative histological studies on the aged human brain. I. Senile plaques and neurofibrillary tangles in "normal patients". Acta Neuropathol (Berl) 16:85–94
16. Glenner GG (1989) The pathobiology of Alzheimer's disease. Ann Rev Med 40:45–51
17. Glenner GG, Wong CW (1984) Alzheimer's disease: initial report of the purification and characterization of a novel cerebrovascular amyloid protein. Biochem Biophys Res Commun 120:885–890
18. Glenner GG, Wong CW, Quaranta V, Eanes ED (1984) The amyloid deposits in Alzheimer's disease: their nature and pathogenesis. Appl Pathol 2:357–369
19. Heyman A, Wilkinson WE, Stafford JA, Helms MJ, Sigmon AH, Weinberg T (1984) Alzheimer's disease: a study of epidemiological aspects. Ann Neurol 15:335–341
20. Hollander D, Strich SJ (1970) Atypical Alzheimer's disease with congophilic angiopathy, presenting with dementia of acute onset. In: Wolstenholme GEW, O'-Connor M (eds) Alzheimer's disease and related conditions. Churchill, London, pp 105–124
21. Ihara Y (1988) Massive somatodendritic sprouting of cortical neurons in Alzheimer's disease. Brain Res 459:138–144
22. Ikeda S, Allsop D, Glenner GG (1989) Morphology and distribution of plaque and related deposits in the brains of Alzheimer's disease and control cases. An immunohistochemical study using amyloid β-protein antibody. Lab Invest 60:113–122
23. Ikeda S, Yanagisawa N, Allsop D, Glenner GG (1990) Early senile plaques in Alzheimer's disease demonstrated by histochemistry, immunocytochemistry and electron microscopy. Hum Pathol 21:1221–1226
24. Joachim CL, Duffy LK, Morris JH, Selkoe DJ (1988) Protein chemical and immunocytochemical studies of meningovascular β-amyloid protein in Alzheimer's disease and normal aging. Brain Res 474:100–111
25. Johnson J (1960) Organic psychosyndromes due to boxing. Br J Psychiatry 115:45–53
26. Kang J, Lemaire H-G, Unterbeck A, Salbaum JM, Masters CL, Grzeschik K-H, Multhaup G, Beyreuther K, Müller-Hill B (1987) The precursor of Alzheimer's disease amyloid A4 protein resembles a cell-surface receptor. Nature 325:733–736
27. Kidd M (1963) Paired helical filaments in electron microscopy of Alzheimer's disease. Nature 197:192–193
28. Kitamoto T, Ogomori K, Tateishi J, Prusiner SB (1987) Formic acid pretreatment enhances immunostaining of cerebral and systemic amyloids. Lab Invest 57:230–236
29. Kowall NW, Kosik KS (1987) Axonal disruption and aberrant localization of tau protein characterize the neuropil pathology of Alzheimer's disease. Ann Neurol 22:639–643
30. Löfberg H, Grubb AO, Nilsson EK, Jensson O, Gudmundsson G, Blöndal H, Arnason A, Thorsteinsson L (1987) Immunohistochemical characterization of the amyloid deposits and quantitation of pertinent cerebrospinal fluid proteins in hereditary cerebral hemorrhage with amyloidosis. Stroke 18:431–440
31. Martland HS (1928) Punch drunk. JAMA 91:1103–1107
32. Masters CL, Multhaup G, Simms G, Pottgiesser J, Martins RN, Beyreuther K (1985) Neuronal origin of a cerebral amyloid: neurofibrillary tangles of Alzheimer's disease contain the same protein as the amyloid of plaque cores and blood vessels. EMBO J 4:2757–2763
33. Mortimer JA, French LR, Hutton JT, Schuman LM (1985) Head injury as a risk factor for Alzheimer's disease. Neurology 35:264–267
34. Papasozomenos SC (1989) Tau protein immunoreactivity in dementia of the Alzheimer type. I. Morphology, evolution, distribution, and pathogenetic implications. Lab Invest 60:123–137
35. Probst A, Anderton BH, Brion J-P, Ulrich J (1989) Senile plaque neurites fail to demonstrate anti-paired helical filament and anti-microtubule-associated protein-tau immunoreactive proteins in the absence of neurofibrillary tangles in the neocortex. Acta Neuropathol 77:430–436

36. Roberts GW (1988) Immunocytochemistry of neurofibrillary tangles in dementia pugilistica and Alzheimer's disease: evidence for common genesis. Lancet ii: 1456–1458
37. Roberts GW, Allsop D, Bruton C (1990) The occult aftermath of boxing. J Neurol Neurosurg Psychiatry 53:373–378
38. Roberts GW, Whitwell HL, Acland PR, Bruton CJ (1990) Dementia in a punch-drunk wife. Lancet 335:918–919
39. Rudelli R, Strom JO, Welch MD, Ambler MW (1982) Post-traumatic premature Alzheimer's disease: neuropathologic findings and pathogenetic considerations. Arch Neurol 39:570–575
40. Spillantini MG, Goedert M, Jakes R, Klug A (1990) Topographical relationship between β-amyloid and tau protein epitopes in tangle-bearing cells in Alzheimer disease. Proc Natl Acad Sci USA 87:3952–3956
41. Strich SJ, Oxon DM (1961) Shearing of nerve fibers as a cause of brain damage due to head injury. Lancet ii: 443–448
42. Tabaton M, Mandybur TI, Perry G, Onorato M, Autilio-Gambetti L, Gambetti P (1989) The widespread alteration of neurites in Alzheimer's disease may be unrelated to amyloid deposition. Ann Neurol 26:771–778
43. Tomlinson BE, Blessed G; Roth M (1968) Observations on the brains of non-demented old people. Neurol Sci J 7:331–356
44. Tomonaga M (1981) Cerebral amyloid angiopathy in the elderly. J Am Geriatr Soc 29:151–157
45. Vinters HV, Gilbert JJ (1983) Cerebral amyloid angiopathy: incidence and complications in the aging brain. II. The distribution of amyloid vascular changes. Stroke 14:924–928
46. Wisniewski HM, Narang HK, Corsellis JAN, Terry RD (1976) Ultrastructural studies of the neuropil and neurofibrillary tangles in Alzheimer's disease and post-traumatic dementia. J Neuropathol Exp Neurol 35:367
47. Yamaguchi H, Hirai S, Morimatsu M, Shoji M, Ihara Y (1988) A variety of cerebral amyloid deposits in the brains of the Alzheimer-type dementia demonstrated by β protein immunostaining. Acta Neuropathol 76:541–549
48. Yamaguchi H, Nakazato Y, Shoji M, Ihara Y, Hirai S (1990) Ultrastructure of the neuropil threads in the Alzheimer brain: their dendritic origin and accumulation in the senile plaques. Acta Neuropathol 80:368–374
49. Yamaguchi H, Nakazato Y, Shoji M, Okamoto K, Ihara Y, Morimatsu M, Hirai S (1991) Secondary deposition of beta amyloid within extracellular neurofibrillary tangles in Alzheimer-type dementia. Am J Pathol 138:699–705
50. Yamamoto T, Hirano A (1986) A comparative study of modified Bielschowsky, Bodian and thioflavin S stains on Alzheimer's neurofibrillary tangles. Neuropathol Appl Neurobiol 12:3–9