Neuroscience Letters, 160 (1993) 139–144

NSL 09827

β-Amyloid precursor protein (βAPP) as a marker for axonal injury after head injury

Stephen M. Gentleman[a,b], Michael J. Nash[a,b], Christopher J. Sweeting[a,b], David I. Graham[c] and Gareth W. Roberts[a,b]

[a]*Serious Mental Afflictions Research Team, Departments of Psychiatry and Anatomy, Charing Cross and Westminster Medical School, London (UK) and* [b]*Department of Anatomy and Cell Biology, St. Mary's Medical School, Imperial College, London (UK) and* [c]*Department of Neuropathology, Institute of Neurological Sciences, Southern General Hospital, Glasgow (UK)*

(Received 1 February 1993; Revised version received 9 April 1993; Accepted 23 June 1993)

Key words: β-Amyloid precursor protein; Axonal injury; Head injury; β-Amyloid; Alzheimer's disease

It has been demonstrated recently that β-amyloid protein (βAP), generally associated with the plaques of Alzheimer's disease, can also be found in the brains of survivors of head injury. In this study the distribution of the βAP precursor protein (βAPP) was examined immunohistochemically to determine if it is colocalized with βAP in such cases. βAPP immunoreactivity was observed in neuronal perikarya in the neocortex and in dystrophic neurites surrounding βAP immunoreactive plaques i.e. in a distribution similar to that seen in Alzheimer's disease. In addition, βAPP immunoreactivity was noted within white matter tracts where it marked damaged axons. However, no colocalisation of βAPP with βAP was observed in any white matter region. These results indicate that processing of βAPP to produce βAP occurs in the synaptic terminal field of axons and illustrate the utility of βAPP immunoreactivity as a general marker for axonal injury.

Alzheimer's disease (AD) is characterised pathologically by the presence of extracellular β-amyloid protein (βAP) deposits, neurofibrillary tangles and neuronal loss [25]. βAP protein is derived from the β-amyloid precursor protein (βAPP) which is a membrane spanning glycoprotein originating from a gene on chromosome 21 [9]. As yet the actual mechanism by which βAPP is processed to βAP is unclear. One hypothesis is that a lysosomal pathway is needed for the production of βAP [6] but more recent evidence has also revealed that βAPP may be cleaved at the amino terminal of the βAP sequence leaving the intact βAP anchored in the membrane [22, 24]. However, in the normal brain βAPP is predominantly cleaved within the βAP sequence to produce a secretory fragment [3]. It has been suggested that increased βAPP expression may lead to an overloading of this pathway resulting in an alternative processing of βAPP and the subsequent deposition of βAP [18].

Recent molecular studies have revealed a cluster of mutations in the βAPP gene of individuals suffering from familial AD [7]. Such mutations are believed to increase the likelihood of βAPP mismetabolism and provide compelling evidence that such an event is central to the development of Alzheimer-type pathology. Whilst these cases of familial AD have proved invaluable in highlighting the central role of βAPP in AD, they account for only some 20% of all AD cases [26]. The remaining 80% of cases are therefore thought to be environmental in origin (or the result of a combination of environmental factors and genetic predisposition).

For many years epidemiological evidence has pointed towards the possibility that head injury may be one such environmental factor [4, 8, 13]. Recent work has begun to establish concrete pathological links between injury to the head and AD, the best evidence being provided by the finding of βAP deposits in approximately 30% of patients with fatal closed head injury [17]. In addition the pathology of dementia pugilistica, or punch drunk syndrome, closely parallels that of AD [16]. Further evidence that neuronal injury may eventually lead to Alzheimer-type changes comes from the results of various animal studies. Increased βAPP mRNA levels are seen following kainate lesions and experimentally induced ischaemia [1, 10]. Based on such results the hypothesis has been put forward that increased βAPP expression may be

Correspondence: S.M. Gentleman, Departments of Psychiatry and Anatomy, Charing Cross and Westminster Medical School, St. Dunstan's Road, London W6 8RP, UK. Fax: (44) 81-846-7025.

part of an acute phase response (the βAPP promoter is sensitive to interleukin-1 (IL-1) and heat shock [19]).

Axonal injury of varying degrees upto and including diffuse axonal injury (DAI) is a common pathological feature of severe non-missile closed head injury [2]. At the microscopic level axonal injury is characterised by swelling of nerve fibres — the 'retraction balls' of Cajal. These 'retraction balls' represent the cut ends of damaged axons [2]. In severe cases the extensive axonal injury is easily visualised in large white matter tracts (e.g. corpus callosum) by silver staining and is known as DAI. DAI has been observed in some of the head trauma cases which have been shown to exhibit post-traumatic βAP deposits [17].

The object of this study was to determine the extent of βAPP immunostaining in sections from the brains of acute head injury patients. Sections were also stained for βAP protein in order to see whether βAPP and βAP deposits were colocalised.

Post mortem brain tissue was obtained from 11 patients with severe head injury; they ranged in age from 23 to 65 years old with survival times ranging from 12 h to 10 days (Table 1). Data from comprehensive histological studies was also available for each case, including the grading of DAI [2]. Some cases had previously been shown to exhibit βAP deposits in one or more brain areas whilst others showed no such immunoreactivity. Sections from temporal and cingulate cortex areas from each case were pretreated with 80% formic acid for 8 minutes and then incubated overnight with a monoclonal antibody to βAPP (22C11, Boehringer) at a dilution of 1:20. They were then processed using an avidin-biotin system as previously described [17]. A set of serial sections were incubated with a monoclonal antibody to βAP (Dako) and processed in the same way.

Sections from a case of neuropathologically confirmed Alzheimer's disease and a case of Down's syndrome were also stained. Sections from 4 patients with no history of neurological disease were used as controls (age range 53–68).

In the head injury cases βAPP immunoreactivity was found in the white matter where it marked areas of axonal damage (Fig. 1A,B), in the perikarya of neurons in the cortical grey matter (Fig. 1C) and in dystrophic neurites surrounding plaques (Fig. 2A). This pattern of immunoreactivity was markedly different from and much more extensive than that seen in age matched controls (Fig. 1D). The controls showed virtually no evidence of axonal staining and staining of perikarya was limited to a faint positivity in isolated neurones.

The extent of axonal damage revealed by βAPP immunostaining varied in degree from small numbers of damaged axons to the extensive axonal damage characteristic of DAI. In all but one of the cases with histologically confirmed DAI the damaged axons were found to be immunoreactive for βAPP. The single negative case was found to differ in the anatomical regions graded for DAI and those immunostained for βAPP. Perikaryal staining was more intense than that seen in the controls and in some cases was as extensive as that seen in the Alzheimer's disease and Down's patients.

βAP immunoreactivity was restricted to vascular de-

TABLE 1

DETAILS OF AGE, SEX, SURVIVAL, AND PRINCIPAL NEUROPATHOLOGICAL FINDINGS FOR PATIENTS STUDIED

F, female; M, male; L, left; R, right; SDH(e), subdural haematoma (evacuated); ACA, anterior cerebral artery; MCA, middle cerebral artery; DAI, diffuse axonal injury; ICP, intracranial pressure. + and − signify presence or absence of fractures, raised ICP, βAP immunoreactive deposits and axonal βAPP immunoreactivity. Number of + provide semiquantitative rating of contusions and DAI.

Case no.	Age	Survival time	Sex	Skull fracture	Haematoma	Contusions	DAI	Ischaemia	Swelling	Raised ICP	Immunoreactivity	
											βAP	βAPP
1	23	5 days	F	−	L SDH(e)	None	−	Diffuse	None	+	−	+
2	24	3 days	F	−	None	None	−	RL A/MCA	RL	+	−	+
3	46	10 days	F	+	R SDH(e)	+	+++	Diffuse	R	+	−	+
4	51	13 h	M	+	R(burst)	++	−	None	R	+	+	+
5	51	1 day	M	+	R SDH(e)	++	−	Diffuse	None	+	+	+
6	53	12 h	M	+	None	++	−	None	L	+	−	+
7	57	1 day	M	+	RL SDH	++	+	Diffuse	RL	+	+	−
8	59	5 days	M	+	R SDH	+	+++	None	None	+	+	+
9	60	1 day	M	+	R SDH	+	+	None	RL	+	+	+
10	61	2 days	M	+	RL SDH(e)	++	−	Diffuse	L	+	−	+
11	65	27 h	M	+	L SDH(e)	++	+++	RL ACA R MCA	R	+	+	+

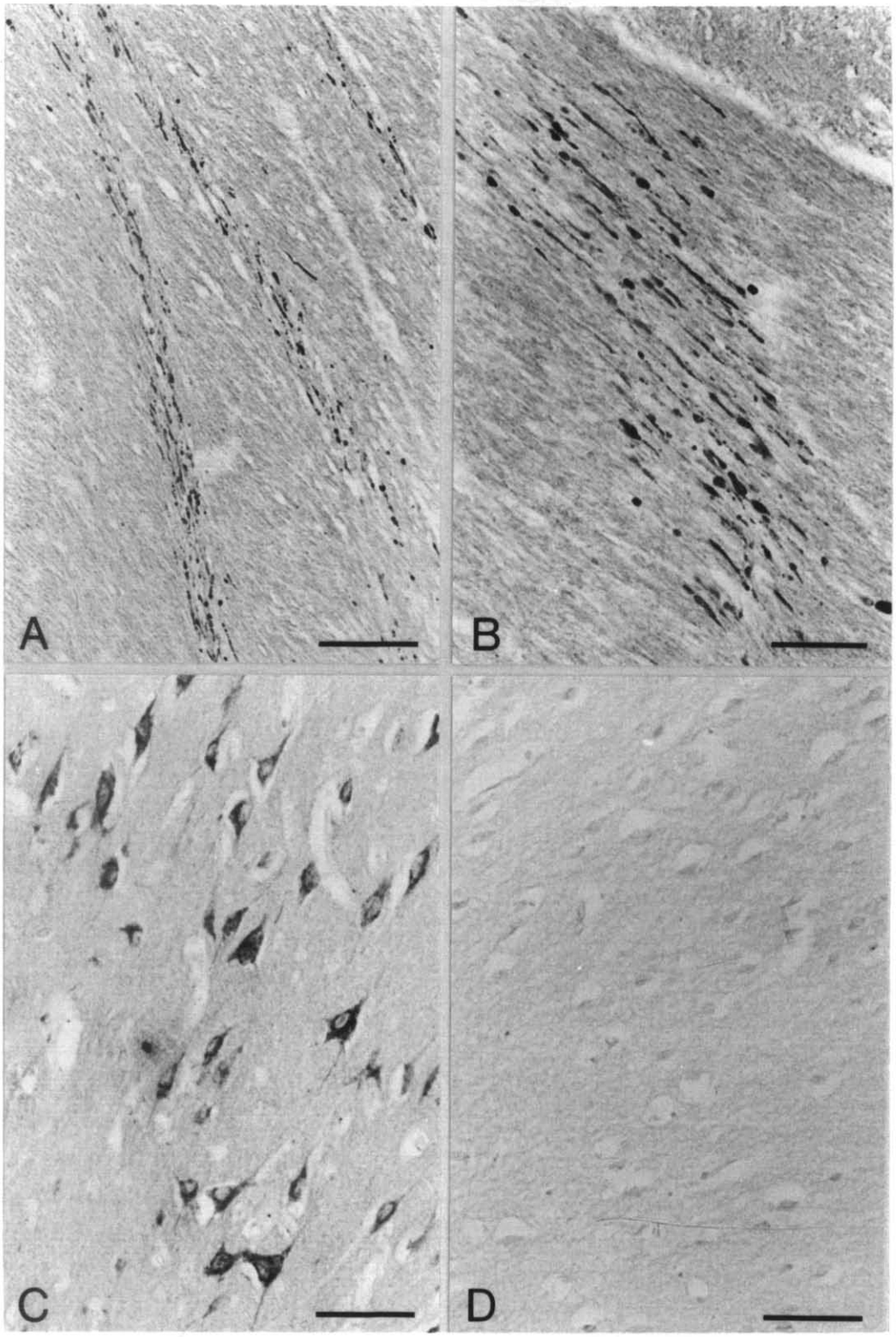

Fig. 1. βAPP immunoreactivity in the white matter of the cingulate cortex (patient 9) marking areas of diffuse axonal injury (A,B). Immunoreactive perikarya in the temporal cortex of a head injured patient (C) as compared to an age matched control (D). Bars = 100 μm (A,C,D), 50 μm (B).

posits, diffuse plaques and the occasional classical plaque (Fig. 2B). Five cases clearly showed βAPP-positive axonal damage in the white matter and the localisation of βAP-immunoreactive deposits in the grey matter within the same cortical region. However, despite colocalisation of βAPP and βAP in some cortical plaques, no

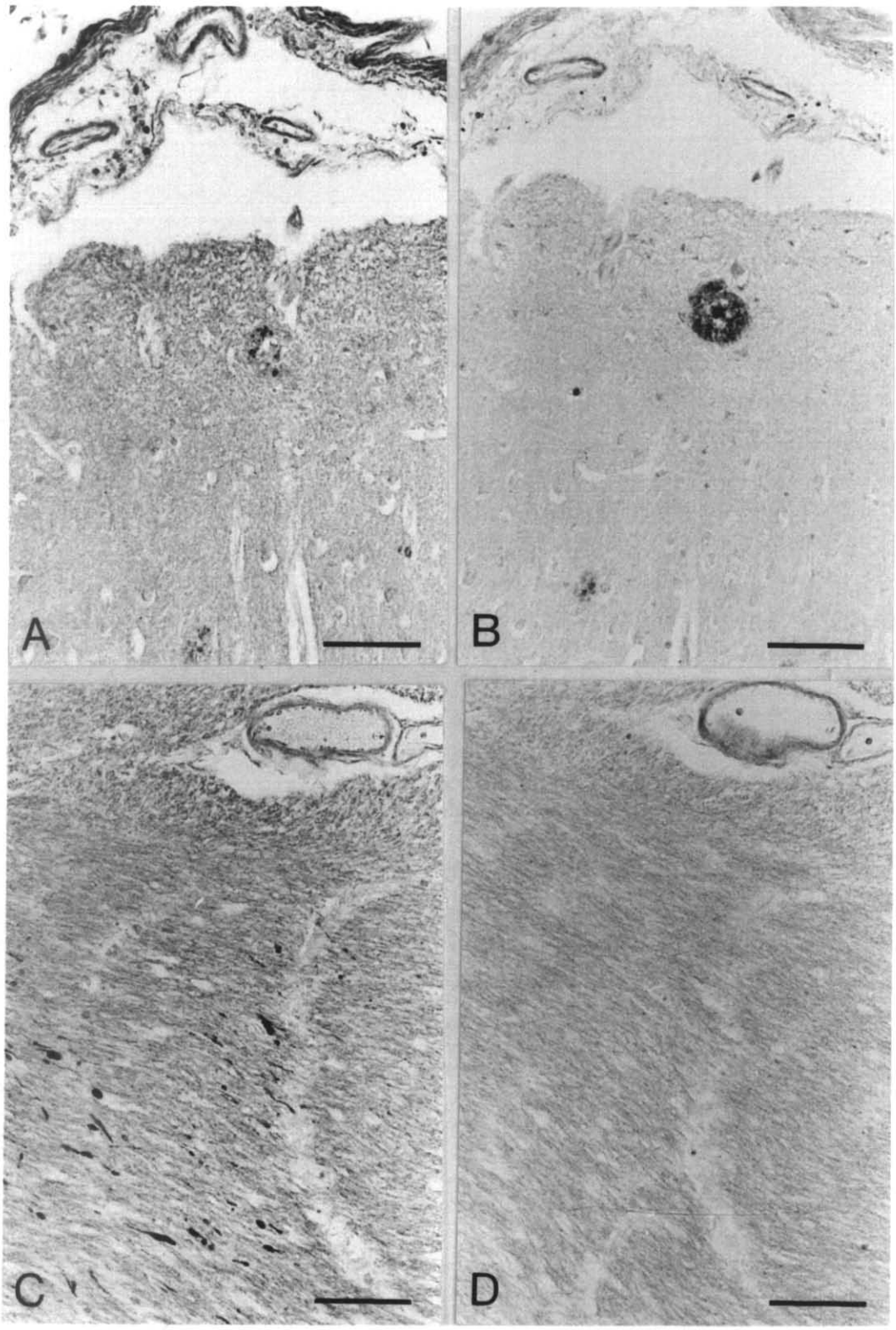

Fig. 2. βAPP immunoreactivity in the cingulate cortex (patient 9) in the dystrophic neurites surrounding a classical plaque (A) and in areas with DAI (C). Serial sections reveal the presence of βAP in classical plaques (B) but not in the areas with DAI (D). Bars = 200 μm (A,B) and 50 μm (C,D).

colocalisation of βAP and βAPP in the white matter was ever observed (Fig. 2C,D).

βAPP appears to be an excellent marker for axonal injury. It was found not only in cases of fatal head injury which we have shown previously to contain cortical βAP deposits but also in cases in which there was no evidence

of βAP having been deposited. An analogous phenomenon has been observed in axonal swellings adjacent to cerebral infarcts [14]. Axotomy of the rat sciatic nerve is also accompanied by increased βAPP in dorsal root ganglion cells [21]. The use of βAPP as a marker for DAI has significant potential in forensic studies since it appears that axonal damage can be detected in cases with short survival times (< 18 h). The standard silver impregnation methods currently in use require survival times in excess of 18 h to reliably demonstrate DAI [2].

βAPP is known to be axonally transported [11] and is located at both pre- and postsynaptic sites [20, 23]. These observations have led to the suggestion that βAPP may have a role in synaptic function and that processing of βAPP may take place at the synapse [20]. Recent studies have suggested that a limited amount of βAPP is processed into βAP during the normal course of cellular metabolism [22, 24]. We have previously suggested that increased local production of βAPP may saturate the normal secretory pathway and result in an increase in βAP production [5]. In some cases this may be sufficient in itself to lead to a βAP accumulation that can reach pathological significance at synaptic sites.

The data from this study provides some of the first indications that these hypothetical events actually occur within the human brain.

It appears that the release of βAPP into the extra-cellular space from injured axons is not sufficient in itself to lead to the production of βAP deposits and that additional factors are required. This observation is of interest in that glial cells are present in both white and grey matter and uptake of βAPP by glial cells has been proposed as an important step in the generation of βAP deposits [27]. However our data indicate that the presence of glial cells is not the critical factor. In order to generate βAP some metabolic process involving the terminal regions of the neurone is needed. Since βAPP is axonally transported it is reasonable to assume that such processing occurs at the synapse [15]. The lack of such machinery at the cut axon ending would explain the presence of βAPP without the presence of βAP.

One may argue that the presence of βAPP in the absence of βAP simply relates to the short time interval between axon damage and brain death, i.e. there was not enough time to generate βAP. However βAP deposits were seen in the cortical grey matter of the same sections in numbers far exceeding those compatible with normal ageing [17]. The existence of these deposits thus goes against this 'time lag' theory.

The subjective impression of increased numbers of βAPP immunoreactive perikarya in the cortex following head injury is consistent with the proposal that this protein may be a component of the brains response to neuronal injury [5, 17]. Our observation has recently been confirmed in a quantitative study which recorded a significant sevenfold increase in the number of cortical βAPP immunoreactive neurons in cases of head injury (in preparation) while other studies also show upregulation of the protein in Alzheimer's disease [12].

The conclusions of this short study are twofold. Firstly it appears that βAPP is an excellent general marker for axonal disruption and further studies are now underway to determine how good it is in relation to conventional silver staining in assessing the extent of axonal damage. Secondly, βAPP and βAP deposits do not colocalise at the sites of axonal injury. βAPP is seen round the damaged axons but βAP is not. This is consistent with other evidence for a synaptic localization for the metabolic machinery needed for the production of βAP from βAPP.

This work was supported by a project grant from the Medical Research Council, UK. S.M.G. is an Alzheimer's Disease Society Research Fellow.

1 Abe, K., Tanzi, R.E. and Kogure, K., Selective induction of kunitz type protease inhibitor domain containing APP mRNA after persistent focal ischaemia in rat cerebral cortex, Neurosci. Lett., 125 (1991) 172–174.
2 Adams, J.H., Head injury. In J. Hume Adams and L.W. Duchen (Eds.), Greenfields Neuropathology, 5th edn., Edward Arnold, London, 1992, pp. 122–132.
3 Anderson, J.P., Esch, F.S., Keim, P.S., Sambamurti, K., Lieberburg, I. and Robakis, N.K., Exact cleavage site of Alzheimer amyloid precursor in neuronal PC-12 cells, Neurosci. Lett., 128 (1991) 126–128.
4 Graves, A.B., White, E., Koepsell, T.D., Reifler, B.V., van Belle, G., Larson, E.B. and Raskind, M., The association between head trauma and Alzheimer's disease, Am. J. Epidemiol., 131 (1990) 491–501.
5 Gentleman, S.M., Graham, D.I. and Roberts, G.W., Molecular pathology of head trauma: altered βAPP metabolism and the aetiology of Alzheimer's disease. In K. Kogure and B.K. Siesjö (Eds.) Progress in Brain Research, Vol. 96, Elsevier, Amsterdam, 1993, pp. 237–246.
6 Haass, C., Koo, E.H., Mellon, A., Hung, A.Y. and Selkoe, D., Targeting of cell-surface β-amyloid precursor protein to lysosomes: alternative processing into amyloid-bearing fragments, Nature, 357 (1992) 500–503.
7 Hardy, J.A., Framing β-amyloid, Nature Genetics, 1 (1992) 233–234.
8 Henderson, A.S., The risk factors for Alzheimer's disease; a review and hypothesis, Acta Psychol. Scand., 78 (1988) 257–275.
9 Kang, J., Lemaire, H.-G., Unterbeck, A., Salbaum, J.M., Masters, C.L., Grzeschik, K.H., Multhaup, G., Beyreuther, K. and Muller-Hill, B., The precursor of Alzheimer's disease amyloid A4 resembles a cell surface receptor, Nature, 325 (1987) 733–736.
10 Kawarabayashi, T., Shoji, M., Harigaya, Y., Yamaguchi, H. and Hirai, S., Expression of APP in the early stage of brain damage, Brain Res., 563 (1991) 334–338.
11 Koo, E.H., Sisoda, S.S., Archer, D.R., Martin, L.J., Weidmann, A., Beyreuther, K., Fischer, P., Masters, C.L. and Price, D.L., Precur-

sor of amyloid protein in Alzheimer's disease undergoes fast anterograde axonal transport, Proc. Natl. Acad. Sci. USA, 87 (1990) 1561–1565.

12 McGeer, P.L., Akiyama, H., Kawamata, T., Yamada, T., Walker, D.G. and Ishii, T., Immunohistochemical localisation of β-amyloid precursor protein sequences in Alzheimer and normal brain tissue by light and electron microscopy, J. Neurosci. Res., 31 (1992) 428–442.

13 Merz, B., Is boxing a risk factor for Alzheimer's disease?, JAMA, 261 (1989) 2597–2598.

14 Ohgami, T., Kitamoto, T. and Tateishi, J., Alzheimer's amyloid precursor protein accumulates within axonal swellings in human brain lesions, Neurosci. Lett., 136 (1992) 75–78.

15 Price, D.L., Aged non-human primates: An animal model of age-associated neurodegenerative disease, J. Cell. Biochem., Suppl., 16E (1992) 200.

16 Roberts, G.W., Allsop, D. and Bruton, C.J., The occult aftermath of boxing, J. Neurol. Neurosurg. Psychiat., 53 (1990) 373–378.

17 Roberts, G.W., Gentleman, S.M., Lynch, A. and Graham, D.I., βA4 amyloid protein deposition in the brain after head trauma, Lancet, 338 (1992) 131–133.

18 Roberts, G.W., Gentleman, S.M., Lynch, A., Murray, L., Landon, M. and Graham, D.I., β-amyloid protein deposition in the brain following severe head injury: implications for the pathogenesis of AD, J. Neurol. Neurosurg. Psychiatry, in press.

19 Royston, M.C., Rothwell, N.J. and Roberts, G.W., Alzheimer's disease: from pathology to potential treatments?, TIPS, 13 (1992) 131–133.

20 Schubert, W., Prior, R., Weidmann, A., Dircksen, H., Multhaup, G., Masters, C.L. and Beyreuther, K., Localisation of Alzheimer β/A4 APP at central and peripheral synaptic sites, Brain Res., 563 (1991) 184–194.

21 Scott, J.N., Parhad, I.M. and Clark, A.W., β-amyloid precursor protein gene is differentially expressed in axotomised sensory and motor systems, Mol. Brain Res., 10 (1991) 315–325.

22 Seubert, P., Vigo-Pelfrey, C., Esch, F., Lee, M., Dovey, H., Davis, D., Sinha, S., Schlossmacher, M., Whaley, J., Swindlehurst, C., McCormack, R., Wolfert, R., Selkoe, D., Lieberburg, I. and Schenk, D., Isolation and quantification of soluble Alzheimer's β-peptide from biological fluids, Nature, 359 (1992) 325–327.

23 Shigematsu, K., McGeer, P.L. and McGeer, E.G., Localization of amyloid precursor protein in selective postsynaptic densities of rat cortical neurons, Brain Res., 592 (1992) 353–357.

24 Shoji, M., Golde, T.E., Ghiso, J., Cheung, T.T., Estus, S., Shaffer, L.M., Cai, X-D., McKay, D.M., Tintner, R., Frangione, B. and Younkin, S.G., Production of the Alzheimer amyloid β protein by normal proteolytic processing, Science, 258 (1992) 126–129.

25 Tomlinson, B.E., Ageing and the dementias. In J. Hume Adams and L.W. Duchen (Eds.), Greenfields Neuropathology, 5th edn., Edward Arnold, London, 1992, pp. 1317–1340.

26 van Duijn, C.M., Hofman, A. and Kay, D.W.K., Risk factors for Alzheimer's disease: A collaborative analysis of case control studies, Int. J. Epidemiol., 20 (1991) S4–11.

27 Wegiel, J. and Wisniewski, H.M., Tubuloreticular structures in microglial cells, pericytes and endothelial cells in Alzheimer's disease, Acta Neuropathol., 83 (1992) 653–658.