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A subset of calretinin-positive neurons are abnormal in Alzheimer's disease

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Abstract The distribution of the calcium-binding protein calretinin was investigated by immunohistochemistry in the hippocampus, the subicular areas, and the entorhinal cortex in patients with Alzheimer's disease and in control subjects. By double immunolabelling, the calretinin immunoreactivity was compared to the immunoreactivity for β/A4 amyloid or for tau proteins. Calretinin-positive neurons were mainly observed in the molecular layer of the gyrus dentatus, the stratum radiatum of the Ammon's horn, and in layers II and III of the entorhinal cortex. The general pattern of calretinin immunoreactivity was conserved in Alzheimer's disease. Calretinin-positive neurons appeared normal in the hippocampus but had a reduced dendritic tree in the entorhinal cortex. Dystrophic calretinin immunoreactive fibres were often observed in the outer molecular layer of the gyrus dentatus and in the CA4 sector in Alzheimer's disease. Most neurons containing neurofibrillary tangles were not calretinin immunoreactive and most senile plaques were not associated with calretinin positive fibres. These results show that entorhinal calretinin-positive neurons are affected in Alzheimer's disease in spite of an absence of systematic association with neurofibrillary tangles and senile plaques.

Key words Calretinin · Calcium-binding protein Alzheimer's disease · Hippocampus · Entorhinal cortex

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Introduction

Neurofibrillary tangles and senile plaques are characteristic histological lesions abundant in the brain of people affected with Alzheimer's disease, the most common cause of dementia in elderly people. Neurofibrillary tangles are made of bundles of abnormal filaments (paired helical filaments, PHF) accumulating in neurons, in abnormal neurites in the neuropil (neuropil threads) and in some dystrophic neurites in the senile plaques. Immunochemical and biochemical studies have demonstrated that the main component of isolated PHF is the microtubule-associated protein tau [5, 26]. Tau proteins play an important role in the stabilisation of the microtubule network and are involved in the morphogenetic events leading to axonal differentiation. Tau proteins present in PHF (PHF-tau) differ from the normal tau isoforms, the best documented of these differences being a state of increased phosphorylation [7, 13, 16]. The phosphorylation of tau affects its ability to trigger the polymerisation of microtubules in vitro and an increased phosphorylation of tau in Alzheimer's disease might underly the loss of microtubules and disturbances of axoplasmic flows observed in neurons containing neurofibrillary tangles. The senile plaques are constituted of an extracellular deposit of amyloid fibres surrounded by dystrophic neurites. This amyloid deposit is composed of a 42-amino acid polypeptide, called β/A4 amyloid peptide [30]. This β/A4 amyloid peptide derives from a larger precursor (the amyloid peptide precursor) which is abundant in neurons.

A disturbance of calcium homeostasis leading to a calcium-mediated neuronal death has been proposed to play a role in Alzheimer's disease. For instance, the activation of calcium-dependent enzymatic pathways might be instrumental in an abnormal processing of tau proteins. An increase in intracellular calcium could lead to the activation of selected kinases or kinase cascades involved in the abnormal or excessive phosphorylation of PHF-tau. The calcium-calmodulin-dependent pro-

tein kinase II has been found to phosphorylate in vitro the serine 405 in bovine tau protein [44]. The same serine was reported to be phosphorylated in PHF-tau [19]; other phosphorylated serines have been identified in PHF-tau and several other kinases are also potential candidates involved in the phosphorylation of tau in vivo. The neuronal death in Alzheimer's disease has also been suggested to result from excessive activation of receptors for excitatory amino acids like glutamate, the neurotoxic effects of glutamate being mediated by an increase in intracellular calcium secondary to the stimulation of *N*-methyl D-aspartate (NMDA) receptors. Another rationale for the role of calcium-mediated neurotoxic effects of glutamate has been suggested in recent studies showing that in neuronal cultures treated with glutamate or calcium ionophores, neurons develop antigenic changes similar to those observed in Alzheimer's disease [31]. In addition β/A4 amyloid, which accumulates in the brain tissue in the disease, was also reported to destabilise calcium homeostasis and to render neurons more sensitive to excitotoxic damage [32].

In view of the potential role played by a disturbance of calcium regulation in the disease, the status of calcium-binding proteins in Alzheimer's disease is of particular interest, since reasonable evidence suggests that these proteins play a role in maintaining calcium homeostasis (for review see [2, 20]). Alternatively, since calcium-binding proteins appear during embryonic brain development in parallel with neuritic outgrowth, far before synaptogenesis, it has been postulated that they might be functionally related to the microtubules [10].

Calretinin is a recently discovered calcium-binding protein [36] which shares extensive homologies with calbindin-D28K [35] but is found in largely separated sets of neurons [33, 38]. Calretinin-positive non-granule and non-pyramidal neurons are present in rodent [1, 17, 33] and monkey [34, 42] hippocampus and entorhinal cortex [37, 42]. Recently the distribution of calretinin-immunoreactive (IR) neurons in the frontal and temporal cortex in Alzheimer's disease has been reported [21].

In this study, we describe the distribution of calretinin-IR neurons and fibres in the hippocampus and the entorhinal cortex of human control subjects and in patients with Alzheimer's disease, i.e. in areas known to be strongly lesioned in Alzheimer's disease. The calretinin immunoreactivity was detected simultaneously with the tau or the β/A4 amyloid immunoreactivity, to investigate to which extent there is an anatomical association between histological lesions and calretinin immunoreactivity.

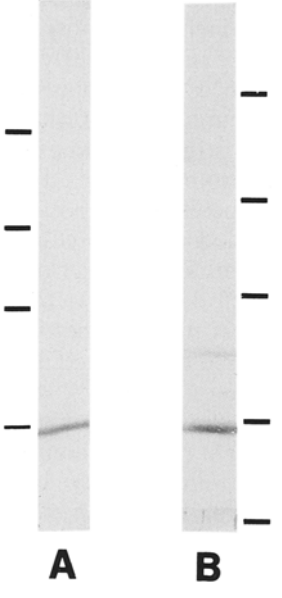

Fig. 1 Western blots of human brain homogenates from a normal subject (**A**) and from a patient with Alzheimer's disease (**B**). The blots, corresponding to two different gels, were incubated with the calretinin antiserum. *Bars* on the *left* and the *right* indicate the position of molecular mass markers: 97.4 kDa (phosphorylase b), 58.1 kDa (catalase), 39.8 kDa (alcohol dehydrogenase), 29.0 kDa (carbonic anhydrase), and 20.1 kDa (trypsin inhibitor)

Materials and methods

Tissue preparation

Tissue blocks including the human hippocampus and the adjacent parahippocampal gyrus were taken at autopsy from two normal subjects (71 and 75 years, post-mortem delays of 12 and 24 h) and from three patients with Alzheimer's disease (57, 75 and 76 years, post-mortem delays of 7, 10 and 20 h). These tissue blocks were fixed for 72 h in 4% paraformaldehyde in phosphate-buffered saline (pH 7.2). Other brain slices were fixed in formalin and embedded in paraffin for neuropathological examination. The patients had a clinical diagnosis of probable Alzheimer's disease and this diagnosis was confirmed by the histological examination showing the presence of numerous neurofibrillary tangles and senile plaques in the hippocampus and in several neocortical areas. The tissue blocks fixed in paraformaldehyde were cut on a Lancer vibratome in tissue sections with a thickness of 30 μm and processed for immunohistochemistry.

Immunocytochemistry

Tissue sections were immunolabelled with a rabbit antiserum to calretinin using the peroxidase-antiperoxidase method as previously reported [6]. Some sections were double immunolabelled with the calretinin antiserum in association either with a mouse monoclonal antibody to β/A4 amyloid or with a mouse monoclonal antibody to bovine tau proteins. For the double immunolabelling, the antibody to calretinin was detected with a goat anti-rabbit antibody (Nordic) followed by a rabbit peroxidase-antiperoxidase complex (Nordic) and diaminobenzidine (DAB) as chromogen, whereas the β/A4 amyloid or tau antibody was detected using a goat anti-mouse antibody conjugated to fluorescein (Sigma). In some experiments, the calretinin and β/A4 amyloid immunoreactivities were demonstrated on the same section using a double-colour technique. The anti-calretinin antibody was detected as above with DAB as chromogen, giving a brown colour; the β/A4 amyloid antibody was detected with the same method (using a mouse peroxidase-antiperoxidase complex) and a mixture of DAB and cobalt chloride as chromogen, giving a blue colour.

The calretinin rabbit antiserum (a kind gift of Dr. J.H. Rogers) has been previously described [36]. It does not cross-react at all with calbindin-D28K [39]. The anti-β/A4 amyloid antibody is a mouse monoclonal antibody prepared using a synthetic peptide (residues 12–28 of the β/A4 amyloid peptide) and was purchased from Boehringer. For the labelling of β/A4 amyloid deposits with the anti-β-amyloid antibody, sections were pretreated with concentrated formic acid [24]. The tau-1 antibody is a mouse monoclonal anti-tau antibody purchased from Boehringer. For optimal labelling of neurofibrillary tangles with the tau-1 antibody, sections were pretreated with alkaline phosphatase, as previously

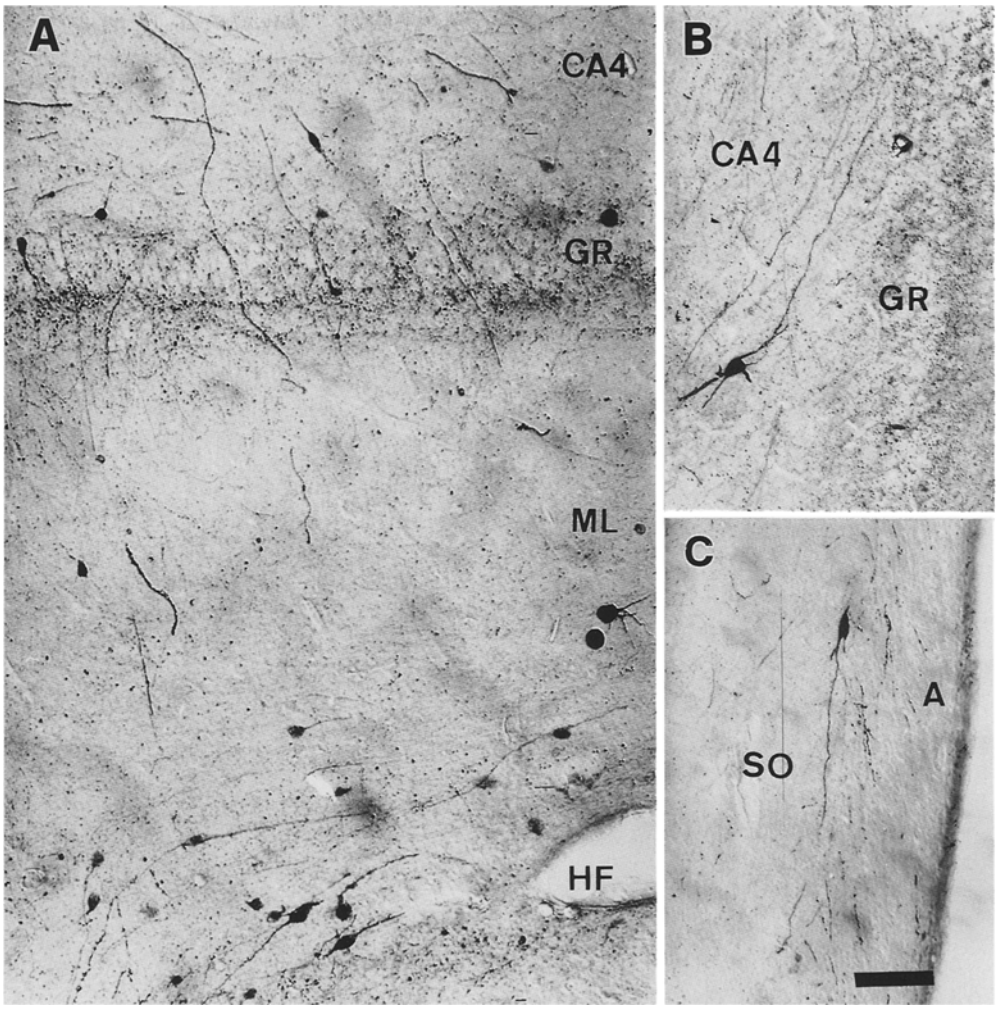

Fig. 2A–C Calretinin immunoreactivity [peroxidase–anti-peroxidase (PAP) method on thick vibratome sections] on coronal sections of the normal human hippocampus. Nomarski optic. **A** Gyrus dentatus. Note the dense fibre labelling in the granule cell layer and the presence of positive fibres originating from neurons in the CA4 sector and crossing the granule cell layer. Calretinin-positive neurons are observed in the external part of the molecular layer. **B** Higher magnification showing a large calretinin-positive neuron in the CA4 sector. **C** Stratum oriens. Some labelled fibres are present in the alveus and labelled neurons are occasionally observed in the stratum oriens (*CA4* CA4 subfield of the Ammon's horn, *GR* granule cell layer, *ML* molecular layer, *HF* hippocampal fissure, *SO* stratum oriens, *A* alveus). *Bar* **A** = 80 μm, **B**, **C** = 100 μm

reported [16]. Control sections were immunolabelled using a non-immune rabbit serum instead of the primary antibodies. Additional sections immunolabelled with the calretinin antiserum were counterstained with Congo red and examined under polarisation filters. In representative sections, calretinin-positive neurons were drawn using a camera lucida and a digitizing tablet.

Western blotting

Human brain extracts were prepared by homogenising tissue samples from the frontal cortex (1 g/10 ml) on ice in 10 mM Tris/HCl, pH 7.4, 0.8 M NaCl, 1 mM EDTA, 10% (w/v) sucrose. The homogenate was centrifuged for 20 min at 15,000 g_{av} and the supernatant retained and kept at –20 °C. Proteins present in the supernatant were separated by electrophoresis on 10% polyacrylamide gels in presence of sodium dodecyl sulphate (SDS-PAGE). After the electrophoresis, proteins were electrophoretically transferred on nitrocellulose sheets. For Western blotting, these nitrocellulose sheets were blocked in semi-fat dry milk (10% w/v) and incubated successively with the calretinin antiserum and a goat anti-rabbit antibody conjugated to alkaline phosphatase (Sigma), as reported [7].

Results

Immunoblotting

The calretinin antiserum labelled only a species of 29–30 kDa on Western blots of brain homogenates of control subjects (Fig. 1A). A band of similar apparent molecular weight was labelled in brain homogenates of patients with Alzheimer's disease (Fig. 1B).

Distribution of calretinin immunoreactivity in the normal hippocampus and entorhinal cortex

The dentate gyrus

Only rare small bipolar cells are calretinin-IR in the dentate gyrus but this layer contains numerous fine varicose fibres surrounding cell bodies (Fig. 2A). These fibres are more numerous in the immediate supragranular zone, constituting a distinct thin band of calretinin fibres. The outer part of the molecular layer contains some medium-sized calretinin-IR neurons; these cells

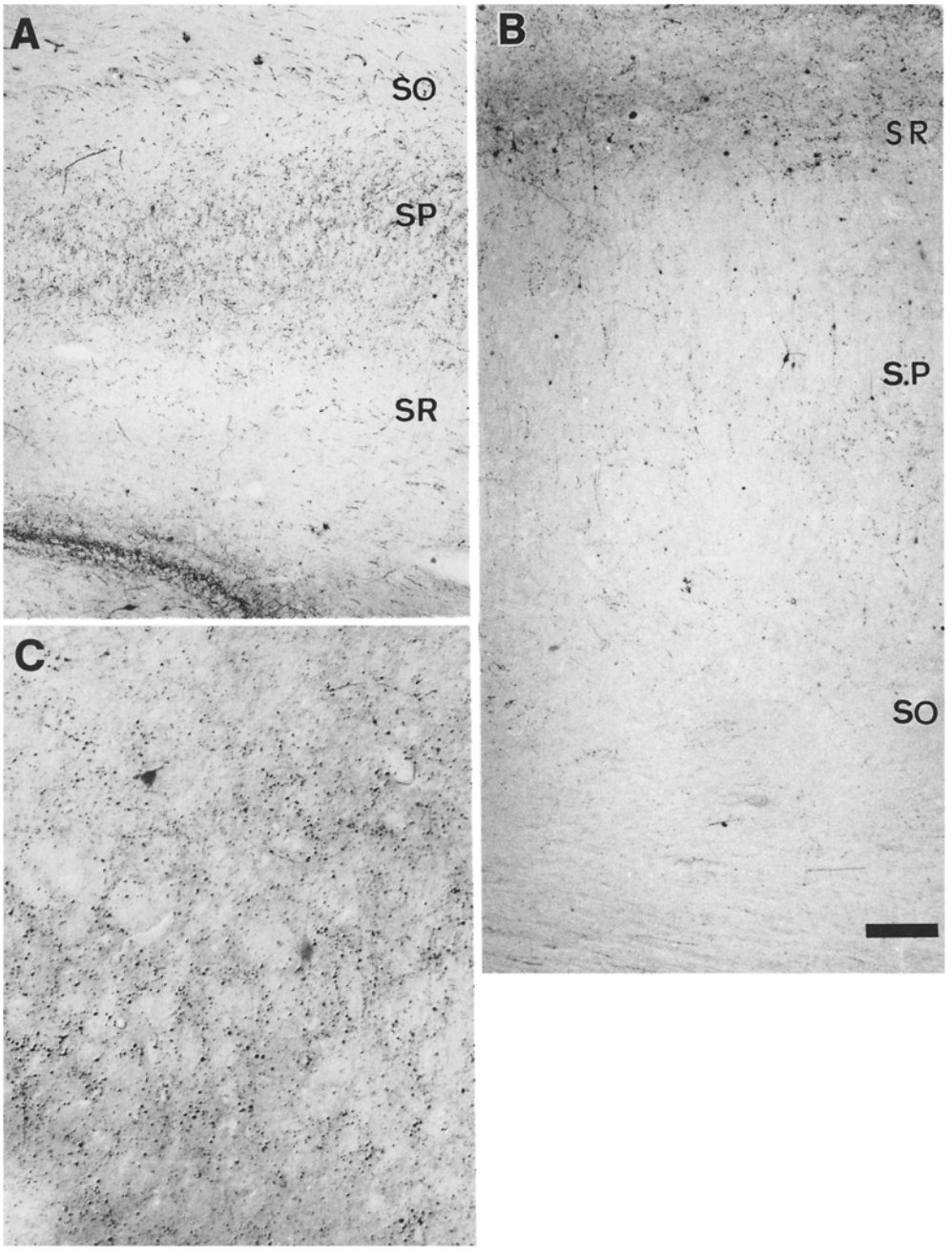

Fig. 3A–C Calretinin immunoreactivity (PAP method on thick vibratome sections) on coronal sections of the normal human hippocampus. **A, C** CA2 sector. An intense calretinin immunoreactivity is present in the stratum pyramidale. As shown at higher magnification in (**C**, Nomarski optic), this immunoreactivity corresponds to numerous boutons and varicose fibres surrounding the pericarya of pyramidal cells. **B** CA1 sector (*SR* stratum radiatum, *SP* stratum pyramidale, *SO* stratum oriens). *Bar* **A, B** = 200 μm, **C** = 25 μm

often have a bipolar shape and are oriented parallel to the obliterated hippocampal fissure. Individual calretinin-positive fibres are present in the molecular layer.

The Ammon's horn

The CA4 sector is the only subfield of the Ammon's horn containing a few large calretinin-IR neurons (Fig. 2B); these multipolar neurons sometimes have one of their processes running perpendicularly through the granule cell layer and into the molecular layer of the dentate gyrus. Large pyramidal neurons in the CA1-CA3 subfields are unlabelled. Small spindle-shaped calretinin-IR neurons, often with processes extending perpendicularly through the stratum radiatum, are relatively numerous in the stratum radiatum and the stratum lacunosum-moleculare. A few small-sized calretinin-IR neurons are observed in the pyramidal cell layer of the CA1 sector. A relatively intense calretinin staining is observed over the stratum pyramidale of CA3 and CA2 sectors (Fig. 3A): this staining is due to numerous fibres and boutons present around the pericarya of CA3 and CA2 pyramids (Fig. 3C). Many calretinin-positive fibres are seen in the stratum radiatum and the stratum lacunosum-moleculare and in lesser abundance in the stratum oriens. Occasionnally, a

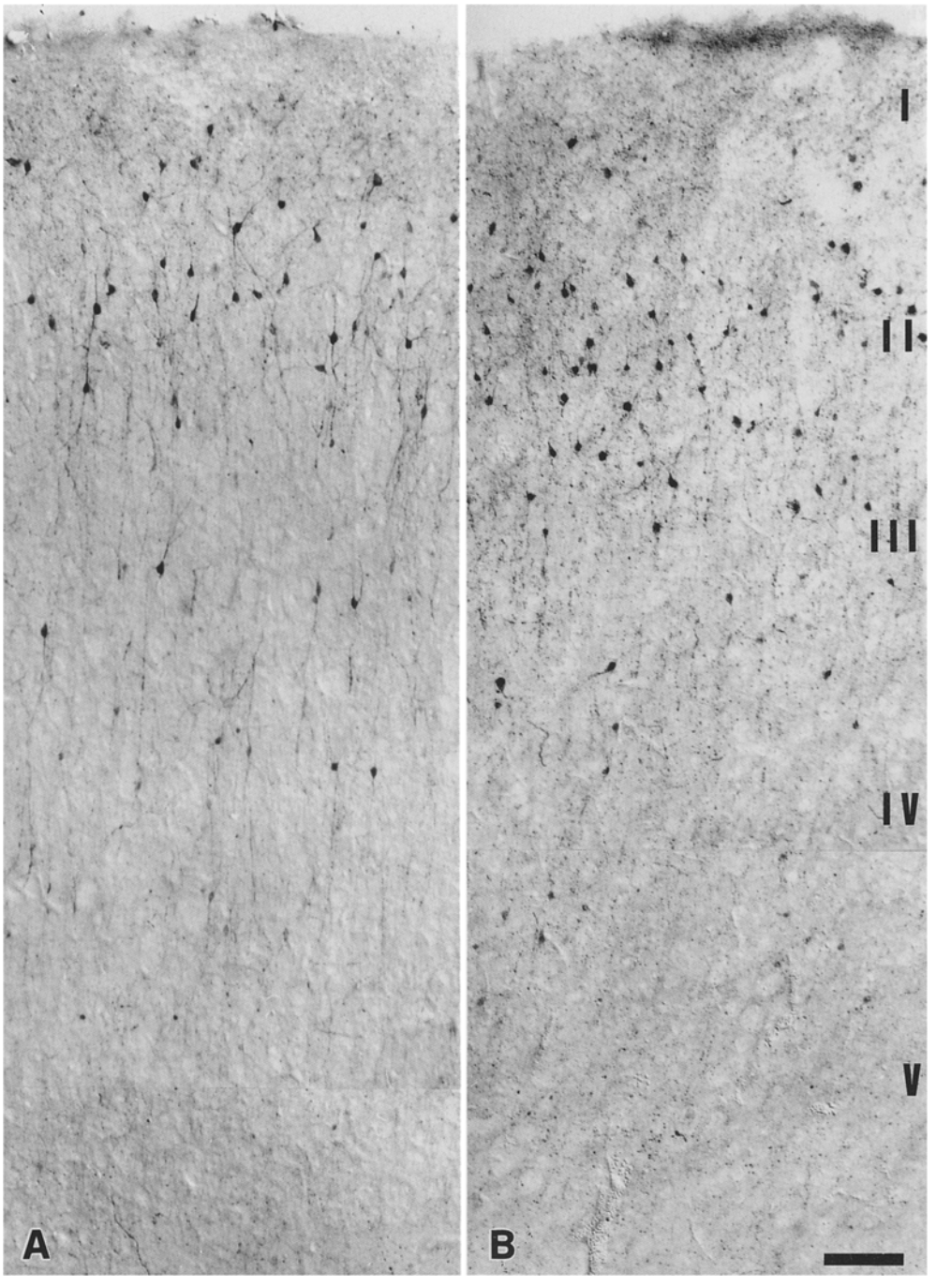

Fig. 4 Calretinin immunoreactivity (PAP method on thick vibratome sections) on coronal sections of the entorhinal cortex in a control subject (**A**) and in Alzheimer's disease (**B**). Nomarski optic. Numerous calretinin-positive neurons are observed in layers II and III of the entorhinal cortex, both in the control and in Alzheimer's disease. In the control these neurons have a bipolar shape with the main dendritic shafts extending perpendicularly to the cortical surface. In Alzheimer's disease, these calretinin-positive neurons show a severe dendritic shortening. The *roman numerals* indicate the position of cortical layers in the entorhinal cortex [28]. *Bar* **A**, **B** = 100 μm

calretinin-IR neuron was present in the stratum oriens (Fig. 2C). A few calretinin-IR fibres were observed in the alveus and in greater abundance in the fimbria.

The subicular complex

A few small-sized calretinin-IR neurons are observed in the pyramidal layers of the subiculum but mainly in the most external part of this layer. These cells have generally the same aspect as in the CA1 sector. Numerous calretinin-IR neurons are present in the layer II of the presubiculum, i.e. in the distinctive islands of cells representing the beginning of this region (presubicular "clouds"; not shown). Calretinin-IR fibres are also abundant in this layer and a few fibres are also seen in the area adjacent to the white matter.

The entorhinal cortex

Calretinin-IR neurons are numerous in layers II and III of the entorhinal cortex (Fig. 4A). Occasional calretinin-positive cells are also found in deeper layers.

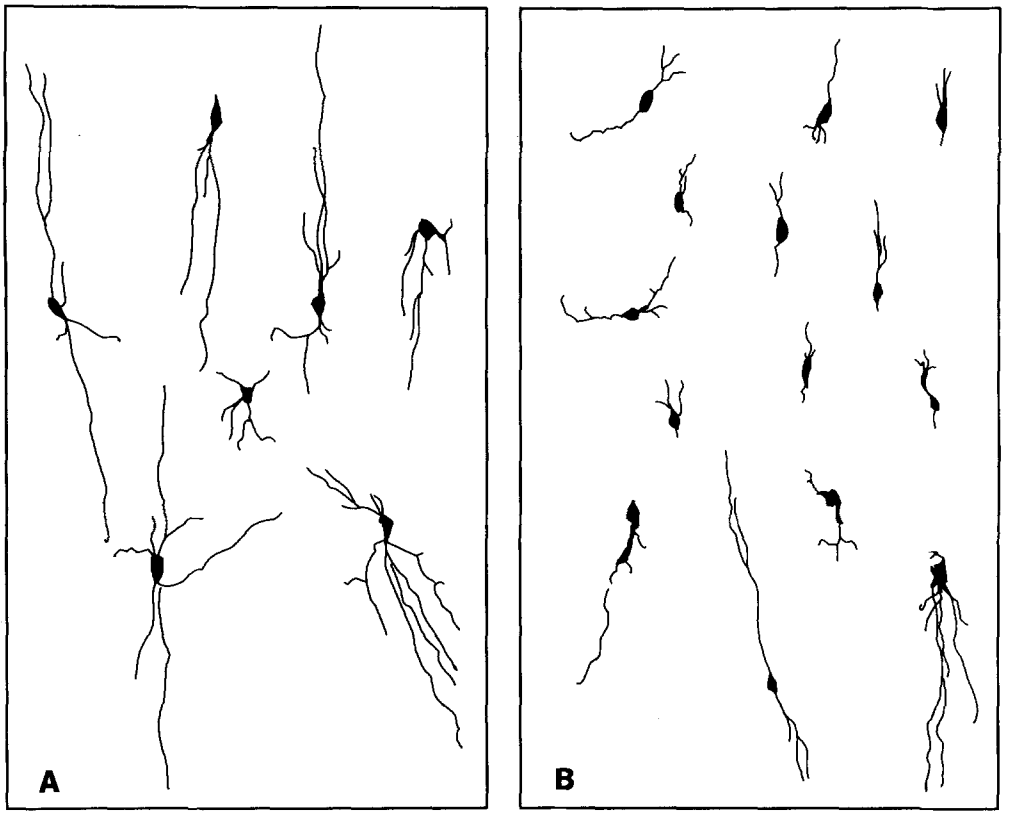

Fig. 5 Camera lucida drawing of a selection of calretinin-positive neurons in the entorhinal cortex of a control subject (**A**) and in Alzheimer's disease (**B**). In Alzheimer's disease, most of these neurons have a severely shrunken neuritic arborization, with much shorter and tortuous neurites

These medium-sized ovoid cells have generally a bipolar shape with long processes extending perpendicularly to the cortical surface (Fig. 5A). Calretinin-IR fibres are present in all layers but are more abundant in layers II and III. The molecular layer also contains numerous calretinin-IR fibres. The white matter of the entorhinal cortex shows occasional calretinin-IR fibres.

Distribution of calretinin immunoreactivity in Alzheimer's disease tissue

The general distribution of calretinin immunoreactivity in the hippocampus and the entorhinal cortex is similar in Alzheimer's disease and in control subjects. Several abnormal calretinin-IR structures are, however, conspicuous in cases of Alzheimer's disease.

The molecular layer of the dentate gyrus, mainly the external part of this layer, contains enlarged, irregular calretinin-IR fibres (Fig. 6A, B). Occasionally, the senile plaques in the molecular layer of the gyrus dentatus are associated with one or several of these dystrophic calretinin-IR fibres (Fig. 6A). Dystrophic fibres are also encountered in other areas, e.g. in the fimbria (Fig. 6C). A few calretinin-IR fibres showing large dilatations are observed in the CA4 sector (Fig. 6D).

Although the number of positive cells appears normal, some calretinin-IR neurons exhibit processes with abnormal shape; these modifications are frequently seen in the entorhinal cortex (Fig. 4B). Such cells have shorter, more tortuous and irregular neurites (Fig. 5B).

Double immunolabelling with the anti-tau antibody or counterstaining with Congo red shows that all neurofibrillary tangle-bearing neurons in the hippocampus are only present in the pyramidal layer and are not calretinin-IR (Fig. 7A, B). In the entorhinal cortex, numerous neurofibrillary tangles are seen in layers II and IV but are not observed in calretinin-IR neurons. In one patient, a few calretinin-IR neurons in layer II of the entorhinal cortex contain neurofibrillary tangles. In this case, many calretinin-positive neurons in this layer have a severely dystrophic shape. This absence of calretinin immunoreactivity in most neurons containing neurofibrillary tangles is also observed in the frontal cortex of Alzheimer's disease patients (not shown). The abundant tau-IR dystrophic neurites dispersed in the neuropil (neuropil threads) are calretinin negative. With the exception of a few plaques in the molecular layer of the gyrus dentatus (see above), most dystrophic neurites in senile plaques are not labelled by calretinin antibodies (Fig. 7B).

Double immunolabelling with the anti-β/A4 amyloid antibody or counterstaining with Congo red show that most of the β/A4 amyloid deposits are not selectively associated with calretinin-IR cells or fibres (Fig. 8A, B). Occasionally, neurites with an apparent normal shape can be observed crossing a β/A4 amyloid deposit (Fig. 8C).

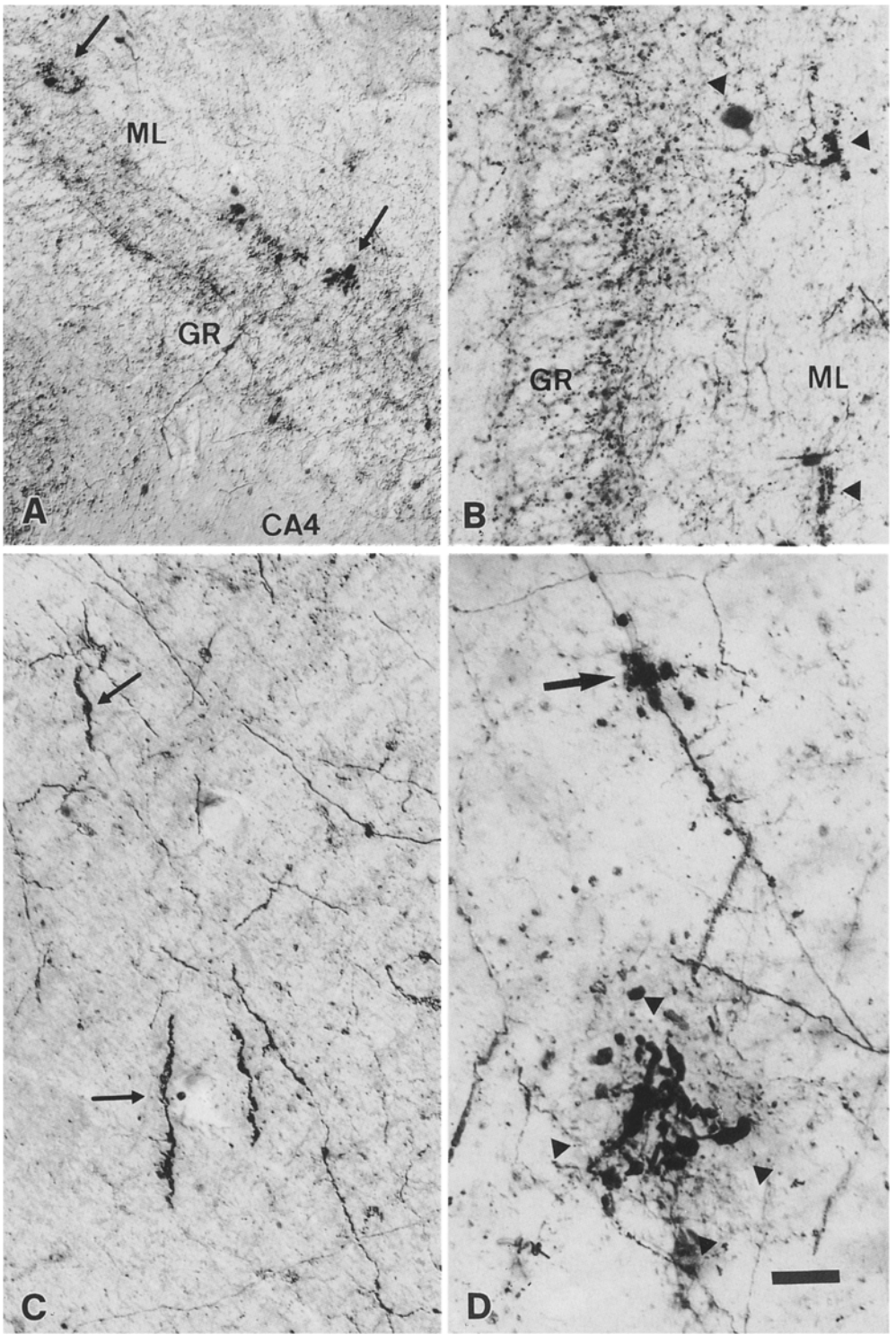

Fig. 6A–D Calretinin immunoreactivity (PAP method on thick vibratome sections) on coronal sections of the hippocampus in Alzheimer's disease. **A**, **B** Gyrus dentatus; **C** fimbria; **D** CA4 sector. **A**, **C**, **D** Nomarski optic. The molecular layer contains numerous dystrophic neurites (*arrowheads* in **B**), sometimes associated with senile plaques (*arrows* in **A**). Dystrophic neurites are also observed in the fimbria (*arrows* in **C**). Some calretinin-positive fibres show focal dilatation (*arrow* in **D**). The *arrowheads* in **D** show calretinin-positive dystrophic neurites associated with a senile plaque. *Bar* **A** = 80 μm, **B** = 50 μm, **C** = 40 μm, **D** = 25 μm

Discussion

This study reports the distribution of calretinin immunoreactivity in the normal human hippocampus and entorhinal cortex and in the same areas in Alzheimer's disease. Calretinin was found to be strongly expressed only in a subset of interneurons and fibres. In the hippocampus, no spiny positive cells were observed. A similar distribution of calretinin-positive cells in the hippocampus was previously reported in monkey [42]. Calretinin-positive cells of the rat hippocampus are different: they are present in all layers and some of them have spiny dendrites [17]. In both rat [33] and monkey [34] calretinin-positive cells are prominently co-localized with γ-aminobutyric acid (GABA). As previ-

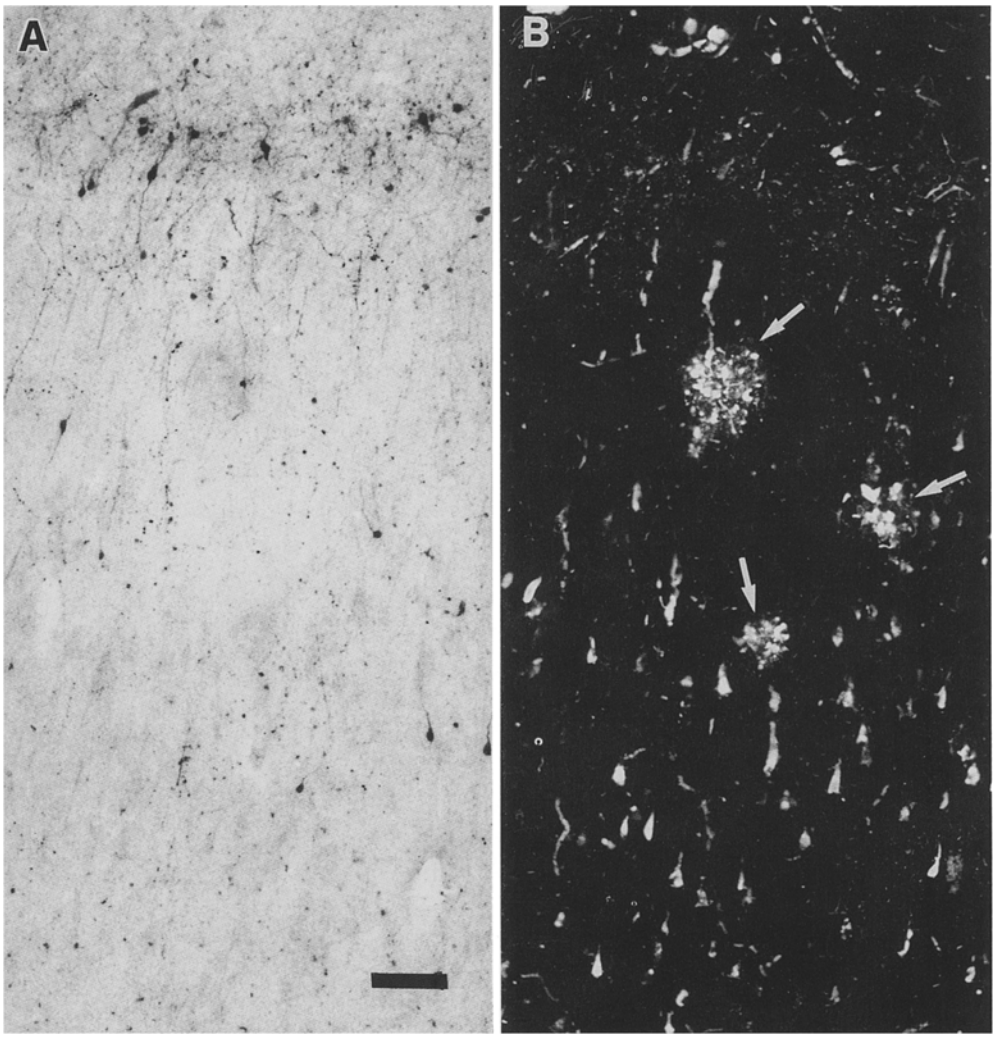

Fig. 7 Double immunolabelling with the calretinin antibody (**A**) and the tau antibody (**B**) on a coronal section (thick vibratome section) of the hippocampus in Alzheimer's disease, CA1 sector. The same field is examined in bright field (**A**) or under ultraviolet illumination (**B**). Numerous neurofibrillary tangles in the pyramidal cell layer are labelled with the anti-tau antibody. Dystrophic neurites in three senile plaques (*arrows* in **B**) are also labelled by the anti-tau antibody but not by the anti-calretinin antibody. *Bar* **A**, **B** = 100 μm

ously reported in monkey [34], but not in rat, a high density of calretinin-IR terminals was observed in the human CA2 sector. In the monkey, these terminals arise from hypothalamic neurons localized in the supramammillary nucleus [34].

Calretinin was also strongly expressed in medium-sized neurons with a bipolar shape and present in layers II and III of the entorhinal cortex. Many of these neurons had the appearance of interneurons. Similar cells were observed in layers II and III of the rat cerebral cortex [23, 39], in the monkey entorhinal cortex [42] and in the human primary visual cortex [15]. None of the normal entorhinal calretinin-positive cell was strictly comparable to the richly ramified cells described by Lorente de No [28]. This was not surprising since calretinin immunoreactivity is not the equivalent of a true Golgi staining.

The entorhinal cortex is severely affected in Alzheimer's disease [4]; numerous neurofibrillary tangles accumulate in layers II and IV which also show severe cell loss and decrease of synaptic markers. The presence of numerous neurofibrillary tangles in layer II in the entorhinal cortex in Alzheimer's disease most probably leads to partial deafferentation of the hippocampus by destruction of the perforant pathway [22]. In experimental ablation of the entorhinal cortex, this deafferentation of the dentate gyrus leads to an enhancement of acetylcholinesterase staining in the molecular layer of the gyrus dentatus [29], as a consequence of the sprouting of cholinergic afferents from the basal forebrain [43]. A similar increase in acetylcholinesterase staining and the presence of acetylcholinesterase-positive senile plaques in the molecular layer of the gyrus dentatus have been reported in some cases of Alzheimer's disease [14] and have been suggested to reflect the occurrence of sprouting in this layer in response to entorhinal lesions. According to these views, it seems plausible to speculate that the presence in the molecular layer of the gyrus dentatus of calretinin-IR neurites in senile plaques is also related to the lesion of the perforant pathway. The origin of the calretinin-IR fibres associated with these senile plaques is, however, not clear from the present data. Similar parvalbumin-IR dystrophic neurites have been found associated with senile plaques in the frontal cortex in Alzheimer's disease [12].

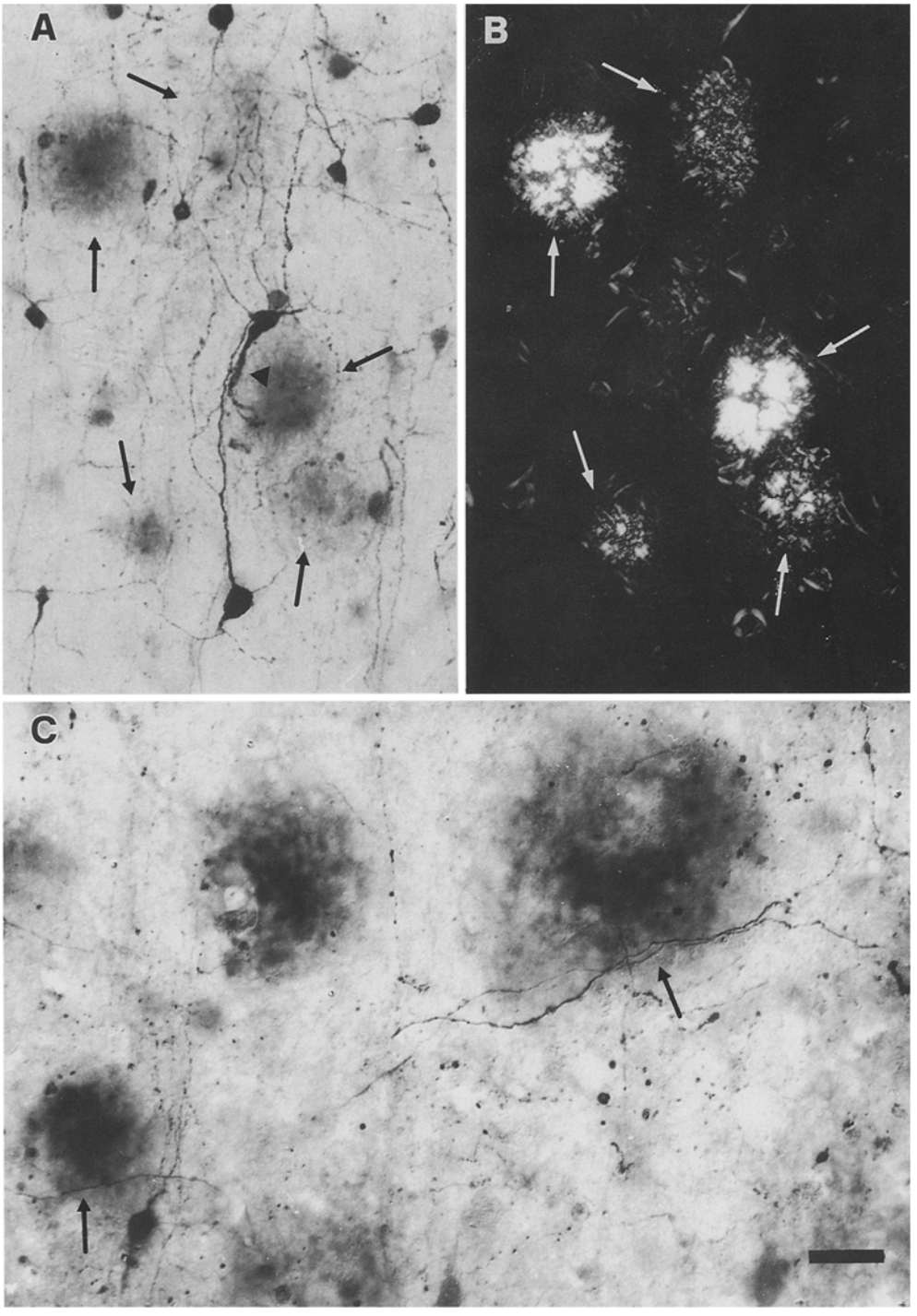

Fig. 8A, B Calretinin immunoreactivity (PAP method on thick vibratome sections) and Congo red staining on a coronal section of the cortex in Alzheimer's disease. **C** Double immunolabelling with the calretinin antibody and the β/A4 amyloid antibody on a coronal section of the hippocampus in Alzheimer's disease, CA1 sector. **A, B** Micrographs of the same field examined in bright field (**A**) or between crossed polarisation filters (**B**). Several calretinin-positive neurons are observed at proximity of β/A4 amyloid deposits in senile plaques (*arrows* in **A** and **B**) which appear in half-tone after Congo red staining. A dilated neurite near a β/A4 amyloid deposit is seen in (**A**, *arrowhead*). Calretinin-positive fibres with normal shape (*arrows* in **C**) are also observed closely adjacent to β/A4 amyloid deposits. *Bar* **A**, **B** = 32 μm, **C** = 25 μm

With the exception of these senile plaques in the molecular layer of the gyrus dentatus, most dystrophic neurites in senile plaques in the hippocampus and the entorhinal cortex were calretinin negative. We more often observed calretinin-IR neurites with normal shapes in the close vicinity of β/A4 amyloid deposits or even crossing these β/A4 amyloid deposits, suggesting that calretinin-IR neurons do not frequently give rise to the neuritic component of senile plaques. Since β/A4 amyloid, especially when aggregated in the form of amyloid fibres, has been reported to be neurotoxic [25], the frequent sparing of calretinin-IR neurons or neurites in contact with β/A4 amyloid deposits suggests that these cells are relatively resistant to the neurotoxic action of β/A4 amyloid.

The calretinin-IR neurons in the hippocampus did not contain neurofibrillary tangles in patients with Alzheimer's disease. This might result from the absence of overlap between the population of neurons specifically susceptible to the formation of neurofibrillary tangles

(pyramidal neurons in the Ammon's horn) and the population of neurons normally expressing calretinin in the hippocampus (e.g. interneurons in the stratum radiatum). In addition, calretinin-IR neurons located in neuronal layers containing numerous neurofibrillary tangles, as in layer II of the entorhinal cortex or layer III of the frontal cortex (personal observation), infrequently contained neurofibrillary tangles.

These immunocytochemical results indicate thus that calretinin-IR neurons are not a cell population specifically susceptible to the formation of neurofibrillary tangles. The expression of calretinin in these neurons could conceivably protect them against the formation of neurofibrillary tangles, e.g. by buffering an intracellular increase of calcium which has been postulated to play a role in the development of neurofibrillary tangles and cell death in Alzheimer's disease [18]. It could be argued that a general down-regulation of protein synthesis in neurons affected by neurofibrillary tangles formation would lead to a disappearance of calretinin in these cells, accounting for the apparent absence of calretinin immunoreactivity in neurofibrillary tangles-bearing cells. This, however, seems unlikely since no major change in the number of calretinin-positive cells was apparent in Alzheimer's disease.

We did not observe a major change in the distribution of the calretinin-positive cells in the hippocampus and the entorhinal cortex in Alzheimer's disease. Our results are in agreement with a recent study showing that the density of calretinin-immunoreactive cells in the prefrontal and temporal cortex in Alzheimer's disease is unchanged as compared to control cases [21].

Calretinin-IR neurons and neurites with dystrophic shapes were nevertheless consistently observed in the entorhinal cortex of patients with Alzheimer's disease, indicating that these cells, if not specifically susceptible to neurofibrillary tangles formation and β/A4 amyloid neurotoxicity, are not totally spared by the disease process. Dystrophic pyramidal and non-pyramidal cortical neurons have been observed in Alzheimer's disease after staining with the Golgi method [11, 41]. Pyramidal neurons of layer II in the parahippocampal gyrus have been reported to exhibit shrunken dendritic trees in Alzheimer's disease [8]; we provide here evidence that calretinin-positive, non-pyramidal neurons in the same area also show atrophic changes in the disease. Dystrophic neurites can also be visualised by immunolabelling with antibodies to different types of neurotransmitters or neuropeptides [3, 27]. Nevertheless, it is not possible to infer from these data alone that the degeneration of calretinin-positive neurons is a primary rather than a secondary phenomenon.

Several previous studies have been devoted to modifications in Alzheimer's disease of two calcium-binding proteins closely related to calretinin, parvalbumin and calbindin-D28K. The neurons containing these proteins form virtually non-overlapping populations [9, 33, 37, 42]. The results of these studies on calcium-binding proteins in Alzheimer's disease (for review see [2, 20]) are difficult to reconcile, but from these surveys and this work it can be concluded that the cortical and hippocampal interneurons containing either calretinin, calbindin or parvalbumin are more resistant to degeneration than other cell types, although this does not exclude that the neuronal processes of these neurons can show dystrophic changes in some brain areas. It still remains to be proved whether the low vulnerability of this type of interneuron is related to the calcium-binding protein present in their cytoplasm.

In conclusion, the distribution of calretinin-IR neurons appears to be relatively unaffected in Alzheimer's disease. The dendritic tree of calretinin-positive neurons was, however, reduced in the entorhinal cortex but not in the hippocampus. These cells do not show a systematic association with neurofibrillary tangles and senile plaques. Calretinin appears, thus, to be an interesting marker for a neuronal population which is relatively spared by these types of lesions but shows nevertheless degenerative changes in selected areas.

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