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Phil. Trans. R. Soc. Lond. B 1997 **352**, 1781-1792
doi: 10.1098/rstb.1997.0162

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Changes in the ageing brain in health and disease

BRIAN H. ANDERTON

Department of Neuroscience, Institute of Psychiatry, De Crespigny Park, London SE5 8AF, UK

SUMMARY

The brains of individuals, who are cognitively normal, show age-related changes that include an overall reduction in brain volume and weight, which are associated with gyral atrophy and widening of the sulci of the cerebral cortex, and enlargement of the brain ventricles. These changes are partly the result of nerve cell loss but accurate estimates of neuronal loss are notoriously difficult to make. Microscopically, there are increasing amounts of the age-related pigment, lipofuscin, granulovacuolar degeneration in neurones, Hirano bodies, variable amounts of diffuse deposits of β-amyloid in the parenchyma, the presence of neurofibrillary tangles mainly confined to the hippocampus and amygdala, and sparse numbers of senile plaques in these brain regions and also in other cortical areas. Of these changes, neurofibrillary tangles and senile plaques are the neuropathological hallmark of Alzheimer's disease in which they are more abundant and widespread. Alzheimer's disease has therefore been regarded as accelerated brain ageing; however, the realization that there is a strong genetic contribution to developing the disease at least implies that it may not be the inevitable, even if frequent, consequence of old age. Understanding the molecular basis of plaque and tangle formation is advancing greatly and is the main focus of research into the cellular and molecular changes observed in the ageing brain.

1. ANATOMICAL CHANGES IN THE BRAIN ASSOCIATED WITH NON-PATHOLOGICAL AGEING

At a gross level, there is a decrease in brain volume and weight in individuals over the age of 60 years. The most obvious changes are gyral atrophy, widening of sulci, increase in ventricular volume and there is a loss of hippocampal volume (figure 1). These changes all occur in individuals who are not cognitively impaired and begin after the age of 50 years with a subsequent progressive 2–3% loss in brain weight per decade. The loss of brain substance with age is both in grey and white matter and, for example, magnetic resonance imaging studies (MRI) have demonstrated that the ratio of grey to white matter varies with age, there being relatively greater loss of white matter over the age of 50 (Harris *et al.* 1994). This observation is interpreted as indicating loss of myelinated axons, the axons having a greater volume than the cell body and dendrites of the neurones that are lost, the latter two neuronal compartments being located in the grey matter (Esiri *et al.* 1997).

There have been many studies of brain ageing in which neuronal numbers have been estimated in different brain regions. Alterations in normal ageing are still controversial and compounded by a variety of technical problems, as well as shrinkage of neuronal cell bodies such that they may not be counted within a particular class of neurones but, nevertheless, are still present (reviewed in Esiri *et al.* (1997)). However, there is a consensus that neurones in certain brain regions are lost with age (those in the hippocampus, cerebral cortex, amygdala being amongst the regions affected), whereas, other neurones in the cerebral cortex show shrinkage without loss and yet other brain regions seem to be spared of shrinkage and neuronal loss (including the nucleus basalis, an area that becomes involved in Alzheimer's disease in which the neurones do show signs of shrinkage and loss) (Chiu *et al.* 1984). Estimates of the degree of neuronal loss vary between reports, but are in the range of 6–25% neuronal loss in the neocortex up to the age of 100 years (Terry *et al.* 1987; Hansen *et al.* 1988). Neuronal loss in the hippocampus seems to be greater, being up to about 50% over the age range of early teens to 85 years (West 1993).

Changes in dendrites and synapses are other putative mechanisms that impair neuronal function. The evidence suggests, however, that there is dendritic growth in the normal ageing brain and that this may be a compensatory mechanism for neuronal loss (Coleman & Flood 1987). On the other hand, loss of synapses (up to 20%) seems to occur in some cortical regions (Masliah *et al.* 1993), but there is evidence that synaptic density in the hippocampus is not reduced with normal ageing in areas that receive input from layer II neurones of the entorhinal cortex where neuronal loss occurs (Lippa *et al.* 1992). This was suggested to be the consequence of sprouting by the remaining neurones, again as a compensatory reaction to neuronal loss.

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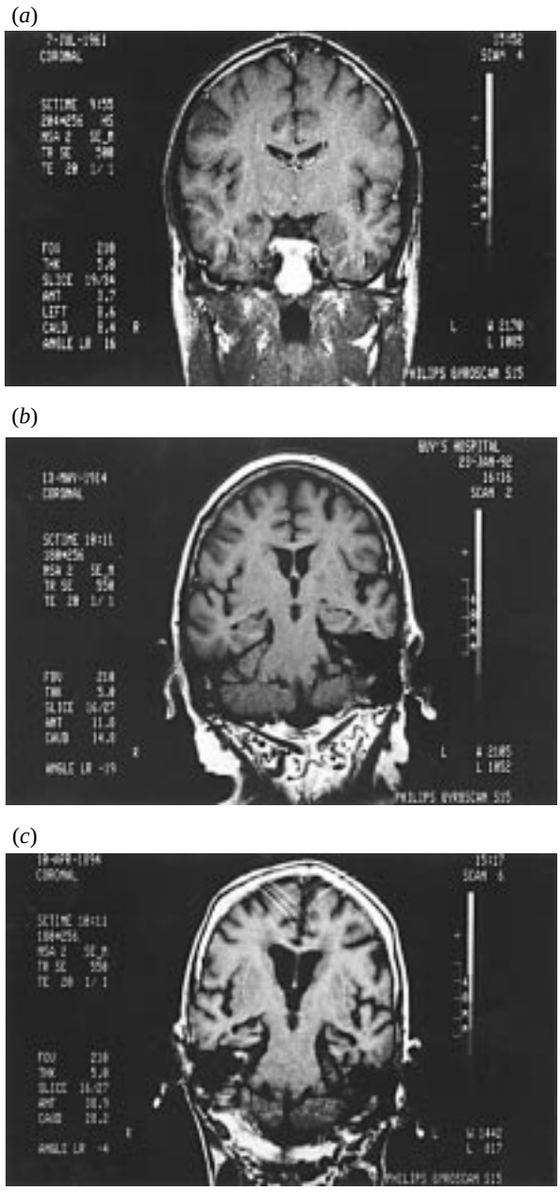

Figure 1. Magnetic resonance images of healthy and Alzheimer's disease human brains. Coronal MRI scans of the brains of (*a*) a healthy 35-year-old male; (*b*) a healthy 82-year-old female; (*c*) a 99-year-old female with Alzheimer's disease.

2. CELLULAR CHANGES

(*a*) *Accumulation of pigment*

Lipofuscin is a pigment that accumulates in some neurones with increasing age. Lipofuscin contains peroxidized protein and lipids, and may represent increasing failure of cells to eliminate these products of peroxidation-induced cell damage. It is not clear if lipofuscin is detrimental to cells since it accumulates in the inferior olive in infancy and there is apparently no loss of these neurones throughout life (Monagle & Brody 1974). Neuromelanin, a related pigment, does appear to be associated with neuronal loss, particularly in the substantia nigra (Mann & Yates 1979).

(*b*) *Neurofibrillary tangles*

Neurofibrillary tangles and senile plaques (see below) are regarded as the histopathological hallmarks of Alzheimer's disease (figure 2). However, they are both present in the brains of non-demented individuals as, indeed, are Hirano bodies and granulovacuolar degenerative changes, the distinguishing feature of Alzheimer's disease being that the numbers of all four features are greater and more widespread. In normal ageing, the numbers of tangles, which occupy the cell body of affected neurones, is therefore relatively low and restricted to the hippocampus, amygdala and entorhinal cortex. However, in a series of brains from demented and non-demented individuals in middle to late life, it has been shown that when minimal neurofibrillary tangles are present, they are found in the transentorhinal region and in brains with increasing neurofibrillary change, the entorhinal cortex is also affected and further pathology includes the hippocampus, with associated cognitive impairment (Braak & Braak 1991). Severely demented individuals are found to have widespread cortical neurofibrillary changes. These observation have led to the proposal that neurofibrillary changes can be staged, with the early stages (I and II) not being associated with dementia.

Ultrastructurally, neurofibrillary tangles are composed of paired helical filaments (PHF) and occasional straight filaments, and studies with biopsy specimens from Alzheimer's patients has shown that in neurones severely affected by PHF, the normal cytoskeleton of microtubules and neurofilaments is totally lost (Flament-Durand & Couck 1979; Gray *et al.* 1987). It is inconceivable that a neurone, or indeed any cell, could survive without a functional cytoskeleton and the presence of ghost or tombstone tangles in postmortem brain specimens with Alzheimer's disease, testifies to neuronal loss. Thus, some neuronal loss is associated with the presence of neurofibrillary tangles in Alzheimer's disease but this may not be a major cause of neuronal loss in normal ageing.

(*c*) *Senile plaques*

Senile plaques are areas of grey matter up to 200 μm across consisting of a central extracellular core of amyloid surrounded by swollen abnormal neurites; these plaques are also known as neuritic plaques (figure 2). The central core contains numerous proteins but the principal protein is a small peptide, 39–43 amino acids long, known as β-amyloid or Aβ, that is aggregated into fibrils. Small numbers of neuritic plaques are present in the normal aged brain and in Alzheimer's disease their numbers are greatly increased. However, large amounts of Aβ are found as 'diffuse deposits' without a neuritic involvement in some brains from individuals who were intellectually intact, although some studies claim that there is a correlation between cognitive decline and total Aβ load in the brain (reviewed in Dickson (1996)). The abnormal neurites surrounding the Aβ core contain numerous PHF and lack normal cytoskeletal structures.

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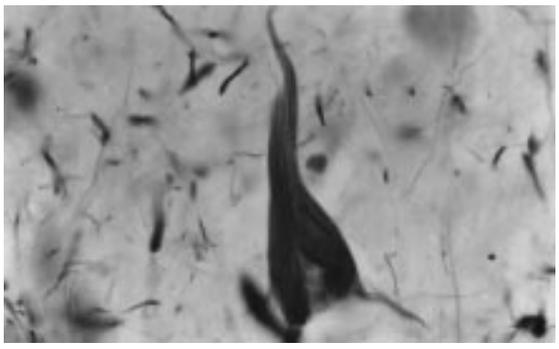
(*a*)



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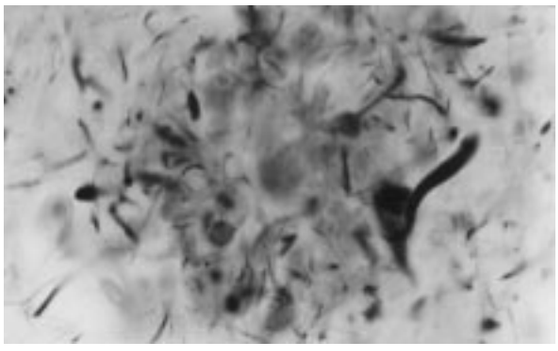

Figure 2. Light microscopy of (*a*) a neurofibrillary tangle in a pyramidal cell (note that the tangle fills the cell body as well as extending into the apical dendrite and the basal dendrites); (*b*) a senile plaque with a pale central core of amyloid surrounded by darkly stained swollen neurites.

(*d*) *Neuropil threads*

Neuropil threads are thread-like structures in the neuropil of the grey matter (Braak *et al.* 1986). Ultrastructurally and immunocytochemically, they have been shown to be essentially the same as the PHF found in neurofibrillary tangles and in the dystrophic neurites of neuritic plaques (Braak *et al.* 1986, 1994). They are therefore another aspect of neurofibrillary degeneration and in normal ageing are restricted mainly to the entorhinal cortex, hippocampus and amygdala, but are more widespread in Alzheimer's disease.

(*e*) *Granulovacuolar degeneration*

As the description implies, the lesion is of one or more apparently empty vacuoles other than for a central granule, which are found in the cell bodies usually of pyramidal cells in the hippocampus. The extent of this lesion increases with age and is more extensive in Alzheimer's disease (Xu *et al.* 1992). Little is known about the biochemical contents of granulovacuoles other than that the granules react with antibodies to cytoskeletal proteins, i.e. neurofilaments, tubulin and tau and also to ubiquitin (Kahn *et al.* 1985; Mayer *et al.* 1989; Dickson *et al.* 1993).

(*f*) *Hirano bodies*

Hirano bodies are rod-shaped structures up to 30 μm long and 8 μm wide that are found in or adjacent to hippocampal pyramidal cells. They increase in number with age and are also more abundant in Alzheimer's disease. Ultrastructurally they appear as paracrystalline arrays of 60–100 nm filaments. Like PHF/straight filaments and granulovacuolar degeneration, Hirano bodies seem to be composed of cytoskeletal proteins but principally those proteins associated with microfilaments, i.e. tropomyosin, α-actinin and vinculin (Galloway *et al.* 1987*a*), although antibodies to tau and neurofilament proteins have also been reported to stain Hirano bodies (Galloway *et al.* 1987*b*; Schmidt *et al.* 1989).

(*g*) *Congophilic angiopathy or cerebral amyloid angiopathy*

This is a change in which there is extracellular deposition of Aβ in the walls of cerebral blood vessels. It is observed with increasing frequency with age and is extensive in Alzheimer's disease. The walls of severely affected blood vessels are thickened and associated with haemorrhage and other vessel wall damage. In rare families with a familial form, massive accumulation of Aβ occurs, resulting in fatal haemorrhage (Wattendorff *et al.* 1995). However, the age-related changes in vessel walls are not just restricted to Aβ deposition since there are also changes in the composition of connective tissue and smooth muscle as well as thickening of the vascular basement membrane (Kalaria 1996).

(*h*) *Infarcts and leucoaraiosis*

The accumulation of numerous small infarcts is possibly the second most common cause of dementia after Alzheimer's disease. Infarcts in small numbers are also found in brains from normal elderly individuals. Leucoaraiosis is a term used to describe a rarefaction of white matter usually close to the ventricles and can be visualized by computed tomography (CT) scanning (Nencini *et al.* 1993). The change tends to be diffuse and is more common in elderly brains and is present in Alzheimer's disease. The cause of the white matter changes are not known but may be the result of partial ischaemia.

3. DEMENTIA—NORMAL OR PATHOLOGICAL AGEING?

The brain, like other organs, is subject to increased incidence of disease as we age; however, it is dementia that is the most common form of pathological ageing and since the severity of dementia is correlated with increased levels of the changes described in the preceding section of this review, the remainder of this article will focus on current knowledge of the biological substrate of dementia, and particularly Alzheimer's disease. It is because the pathological changes of Alzheimer's disease are present in small numbers in the brains of intellectually intact individuals that the question has arisen of whether Alzheimer's disease is simply the inevitable outcome of normal ageing or if it is a disease. There is, as yet, no straightforward answer

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and, indeed, increasingly the question is probably over-simplistic in the light of newly discovered genetic risk factors.

Early onset Alzheimer's disease is usually an autosomal dominant condition and, so far, the identified loci are mutations in the gene for the precursor protein from which Aβ is derived, the amyloid precursor protein (APP), and in the genes for two related proteins, presenilin 1 (PS1) and presenilin 2 (PS2) (table 1). Mutations in APP account for perhaps 5% of familial Alzheimer's disease and mutations in *PS1* for 60–70%; *PS2* mutations are rare and so there is likely to be at least one more gene to be identified, mutations in which would cause autosomal dominant Alzheimer's disease.

Most Alzheimer's disease is late onset but it is now clear that much late onset Alzheimer's disease is associated with possession of the apolipoprotein ε4 allelic form of the apolipoprotein (Roses 1996). There are three common alleles of apolipoprotein E, ε2, ε3 and ε4, with ε3 being the most frequent followed by ε4 and, lastly, ε2, which is relatively rare. Numerous studies of different ethnic groups have shown that the ε4 allele is 2.5 times more frequent in Alzheimer's patients than in the general population and possession of two ε4 alleles results in an earlier age of onset than one ε4 allele, whereas, no ε4 alleles is associated with the latest onset. However, apolipoprotein ε4 is not essential for development of Alzheimer's disease since cases homozygous for apolipoprotein ε3 are not uncommon.

It has also been reported that a polymorphism in the *PS1* gene is associated with increased risk of late-onset Alzheimer's disease (Wragg *et al.* 1996; Kehoe *et al.* 1996), and it is highly likely that other genetic factors will be found to contribute risk. The risk of developing Alzheimer's disease is therefore probably determined by an interaction of the individual's genetic makeup with environmental factors, one example being head injury, which seems to be a risk factor (Schofield *et al.* 1997). Another putative environmental risk factor is herpes simplex virus since a recent study has found that possession of an apolipoprotein ε4 allele only increases risk of Alzheimer's disease in those individuals with post-mortem evidence of having had a latent herpes infection in the brain (Itzhaki *et al.* 1997); it is important that other laboratories confirm this observation because it would clearly have implications for understanding molecular mechanisms and possibly for therapy. Healthy or pathological ageing, more generally, is likely to be determined by this interplay between genotype and environment, as indeed will, perhaps, many if not most diseases, and within the next decade it should be possible by association studies to identify which genes and their alleles contribute risk to disease and confer predisposition for healthy ageing.

Table 1. *Genetic factors in Alzheimer's disease and their biological consequences*

type of genetic effect	biological consequences
(*a*) *autosomal dominant mutations*	
APP_{717}—three mutations of valine to isoleucine, glycine or phenylalanine	increase in the ratio of $A\beta_{42} : A\beta_{40}$
$APP_{670,671}$ double mutation	five to eight-fold increase in total Aβ
presenilin-1 mutations	some demonstrated to increase the ratio of $A\beta_{42} : A\beta_{40}$
presenilin-2 mutations	some demonstrated to increase the ratio of $A\beta_{42} : A\beta_{40}$
one or more genes yet to be identified	?
(*b*) *genetic risk factors*	
apolipoprotein ε4	promotes Aβ aggregation? Fails to protect against tau hyperphosphorylation?
presenilin-1 polymorphism	unknown
other genetic risk factors are likely to be discovered	?

4. THE PATHOGENESIS OF SENILE PLAQUES AND NEUROFIBRILLARY TANGLES

Naturally, research effort into the molecular and cell biology of these lesions has concentrated on their development in Alzheimer's disease but since plaques and tangles in normal brain are indistinguishable from those in Alzheimer brain, the mechanisms underlying their formation are likely to be similar. These same lesions are also present in the brains of those affected by Down's syndrome but they develop at an earlier age, such individuals having abundant plaques and tangles in their fourth decade. However, examination of the brains from younger Down's syndrome cases has shown that diffuse Aβ deposits occur in the late teens but neuritic plaques and tangles do not appear until 20 years later (Mann & Esiri 1989). This observation is a powerful argument for Aβ deposition being an early event in pathogenesis and the presumed mechanism is that there is over-expression of the *APP* gene since it is located on chromosome 21, these individuals therefore having three copies of the gene. Thus, either over-expression of *APP* or expression of mutant forms of *APP* is a likely cause of Aβ production and deposition and recent experimental studies are consistent with such a mechanism, as discussed below.

(*a*) *The amyloid precursor protein (APP) and production of Aβ*;

APP is encoded by a single gene but alternative splicing gives rise to at least eight isoforms, all, apart from one, being transmembrane proteins (Selkoe 1994, 1996) (figure 3). The most common are APP_{695}, APP_{751} and APP_{770}, the latter two possessing one or two extra domains in the extracellular portion of the molecule. One of these domains present in both APP_{717} and APP_{770} is a protease inhibitor known as the Kunitz protein inhibitor (KPI) domain. Cell surface APP has the majority of the molecule as an extracellular domain with a short cytoplasmic tail. The Aβ segment is

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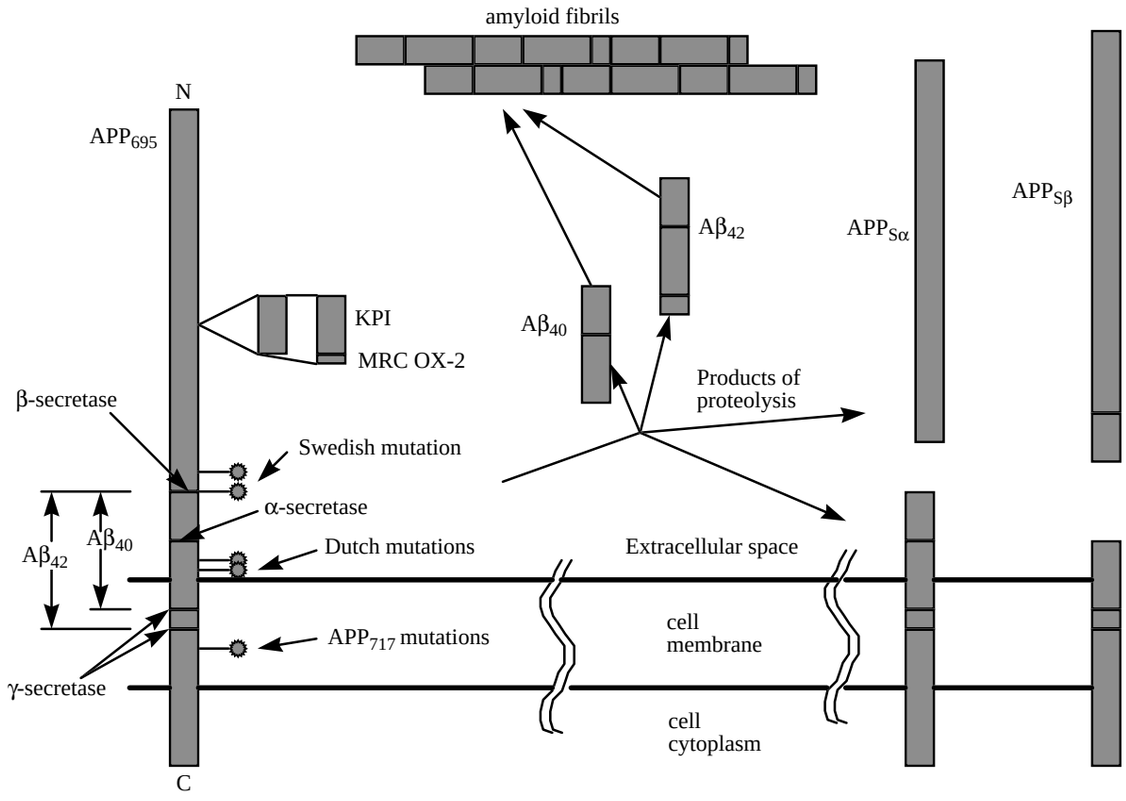

Figure 3. The structure and processing of APP. APP is a transmembrane protein shown here as APP_{695}; APP_{751} includes the KPI domain and APP_{770} has both the KPI and MRC OX-2 domains. The locations of known mutations and secretase cleavage sites are shown, as are the main proteolytic products.

located at the interface of the extracellular domain and the membrane-spanning segment.

The normal function in the brain of APP is unknown although it is expressed by a variety of cells and in platelets the forms with KPI appear to play a role in haemostasis by inhibiting factor XIa (Smith *et al.* 1990). APP has, however, a rapid turnover and is proteolytically processed at several different sites (figure 3). One cleavage site is the so-called α-secretase site which is located within the Aβ sequence. Cleavage at this site is referred to as non-amyloidogenic processing since full-length Aβ cannot be generated by this pathway. Cleavage of APP at the N- and C-termini generates Aβ and these sites are known as β and γ-secretase sites. However, they are not unique sites since Aβ of different lengths can be produced, with species of 40 and 42 amino acids being the most abundant and/or important. Various studies have shown that minor species of Aβ exist that are 'ragged' at both ends, giving rise to molecules that begin both N- and C-terminal of the main β-secretase site and, similarly, that end both N- and C-terminals of the main γ-secretase site.

Mutations in APP that give rise to autosomal dominant Alzheimer's disease flank the Aβ segment (Selkoe 1996). The first genetic lesions to be identified comprising three different point mutations are all located a few residues downstream of the γ-secretase site at position 717 of the APP_{770} species (figure 3 and table 1). A double mutation in a Swedish pedigree is in the two amino acids immediately N-terminal to the main β-secretase site. There are also a few Dutch and Flemish pedigrees in which mutations within the Aβ segment at residues 692 and 693 give rise to massive amyloid angiopathy either without plaques and tangles or with these typical Alzheimer changes; these individuals frequently succumb to a fatal haemorrhage. It is assumed therefore that all of these mutations affect the proteolytic processing of APP at the three secretase sites, and indeed experimental studies using transfected cells has shown that the Swedish double mutation results in a 5–8 fold increase in secreted Aβ (Cai *et al.* 1993) and the APP_{717} mutations result in an increase in the ratio of $A\beta_{42}:A\beta_{40}$ (Suzuki *et al.* 1994). Since Aβ has a tendency to aggregate and because $A\beta_{42}$ has two additional hydrophobic amino acids compared to $A\beta_{40}$, then either a rise in total concentration of Aβ or relatively more of the more hydrophobic $A\beta_{42}$ species can be predicted to drive aggregation.

Another major product of APP processing is the large extracellular domain, APP_S, that is secreted by cells. Depending upon whether this is derived by cleavage at the α- or β-secretase sites, at least two different species are produced (figure 3). APP_S has been found to have neuroprotective properties towards neurones exposed to a variety of insults, including excitotoxic levels of glutamate, and it has been reported that

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$APP_{S\alpha}$ is more potent as a neuroprotectant than $APP_{S\beta}$ (Furukawa *et al.* 1996). This is a putative alternative mechanism to Aβ deposition and neurotoxicity (see below) as the initiating event in Alzheimer's pathogenesis in that changes in the levels of these two forms of APP_S could render neurones more vulnerable to other insults that might trigger subsequent changes in the brain resulting in Alzheimer's disease.

The subcellular sites in which APP is proteolysed are not established with certainty and may vary between cell types. Certainly, some APP is cleaved at the α-secretase site at the cell surface but this is not the only location for this proteolytic event. Figure 4 illustrates schematically the current understanding of the trafficking and metabolism of APP from which it can be seen that some APP is processed to Aβ and APP_S in the endosomal compartment (Yamazaki *et al.* 1996; Koo *et al.* 1996).

Transgenic animal studies have also demonstrated the importance of Aβ for senile plaque formation. Most notably, mice transgenic for an *APP* minigene with one of the APP_{717} mutations develop neuritic plaques remarkably similar to those typical of Alzheimer's disease (Games *et al.* 1995). However, these animals have so far not developed neurofibrillary tangles and so they appear to be a model for only part of the Alzheimer pathology (Masliah *et al.* 1996).

Experimental studies mainly using cultures of neurones have demonstrated that Aβ is neurotoxic but this property is dependent on the aggregation state of Aβ, fibrillar forms being more neurotoxic than amorphous Aβ (Busciglio *et al.* 1995; Yankner 1996; Mattson & Rydel 1996; Fraser *et al.* 1997; Iversen *et al.* 1995; Davis 1996). Since Aβ accumulates as diffuse deposits in the brains of young Down's syndrome patients and also in the non-demented elderly, there is still much to be learned about the properties of different forms of Aβ since in these examples of Aβ deposition in human brain, there is not likely to be significant neurotoxicity since symptoms of dementia are absent.

(*b*) *The presenilins*

The presenilins are serpentine membrane proteins traversing the membrane eight times (figure 5). They are located intracellularly mainly in the endoplasmic reticulum and Golgi apparatus and are proteolytically processed into at least two fragments, cleavage occurring in the large loop between two of the membrane-spanning segments (Doan *et al.* 1996; De Strooper *et al.* 1997). In excess of 35 mutations have been described in PS1 in over 50 familial Alzheimer pedigrees; by contrast only two mutations have been found in PS2 (Hardy 1996).

Presenilin function is unknown but presenilins are homologous to two proteins, Sel-12 and Spe-4, in *C. elegans* (Sherrington *et al.* 1995; Levitan & Greenwald 1995; Levitan *et al.* 1996). The greatest homology is with Sel-12, which has been demonstrated on the basis of genetic experiments to have a role in Notch signalling. Notch is important in cell fate determination in development but must have a role in the adult since it is expressed in adult tissues. Spe-4 is involved in membrane trafficking in spermatids of *C. elegans*. These facts, so far, have not proved to offer much insight into how mutant presenilins trigger the pathogenesis of Alzheimer's disease. However, experimental studies have shown that they may influence APP metabolism because fibroblasts from individuals with presenilin mutations secrete relatively more $A\beta_{42}$ compared to $A\beta_{40}$, as do cells transfected with mutant presenilins, and plasma from Alzheimer's patients possessing mutant presenilins also has relatively more $A\beta_{42}$ as do the brains from transgenic mice expressing such mutant presenilins (Scheuner *et al.* 1996; Duff *et al.* 1996; Borchelt *et al.* 1996). One view concerning the mechanism by which presenilins affect APP metabolism is that they may regulate trafficking of APP through internal membraneous compartments and that this results in changes in the proportion of APP cleaved by different γ-secretases, there being some evidence for the existence of multiple γ-secretases that may be located in different endosomal compartments (Citron *et al.* 1996).

(*c*) *Paired helical filaments (PHF)*

The main structural protein in PHF is the microtubule-associated protein tau. PHF-tau is in a hyperphosphorylated state compared to control postmortem brain tau, with up to 19 phosphates per molecule in PHF-tau. Many of the sites that are phosphorylated in PHF-tau are serine or threonine followed by a proline in the tau sequence (Morishima-Kawashima *et al.* 1995). Our laboratory was one of two first to identify independently glycogen synthase kinase-3 (GSK-3) as a kinase capable of generating *in vitro* a phosphorylation state of tau similar to PHF-tau, as assessed by a marked decrease in electrophoretic mobility and reactivity with a panel of monoclonal antibodies specific for phosphorylated epitopes present in PHF-tau, but not found in normal postmortem brain tau (Mandelkow *et al.* 1992; Hanger *et al.* 1992). Subsequently, it was demonstrated that in transfected cells both forms of GSK-3 (α and β—separate gene products) generate a similar PHF-like phosphorylated state of tau, whereas mitogen-activated protein (MAP) kinase that phosphorylates tau *in vitro* to a state like PHF-tau do not seem capable of generating this phosphorylated state in cells (Lovestone *et al.* 1994; Latimer *et al.* 1995).

(*d*) *Tau phosphorylation is a critical process not only in neurodegeneration but also in neurodevelopment and the role of GSK-3β*

We and our collaborators (Brion *et al.* 1993) were also among the first to demonstrate that in the foetal brain, a proportion of tau is in a hyperphosphorylated state similar to PHF-tau, as judged by immunoreactivity with a panel of phosphorylation-sensitive monoclonal antibodies (Watanabe *et al.* 1993; Kenessey & Yen 1993; Hasegawa *et al.* 1993). Subsequently, it was shown that adult brain tau, when rapidly isolated, also contains a fraction of tau that is in a similarly hyperphosphorylated state (Matsuo *et al.* 1994). However, based upon

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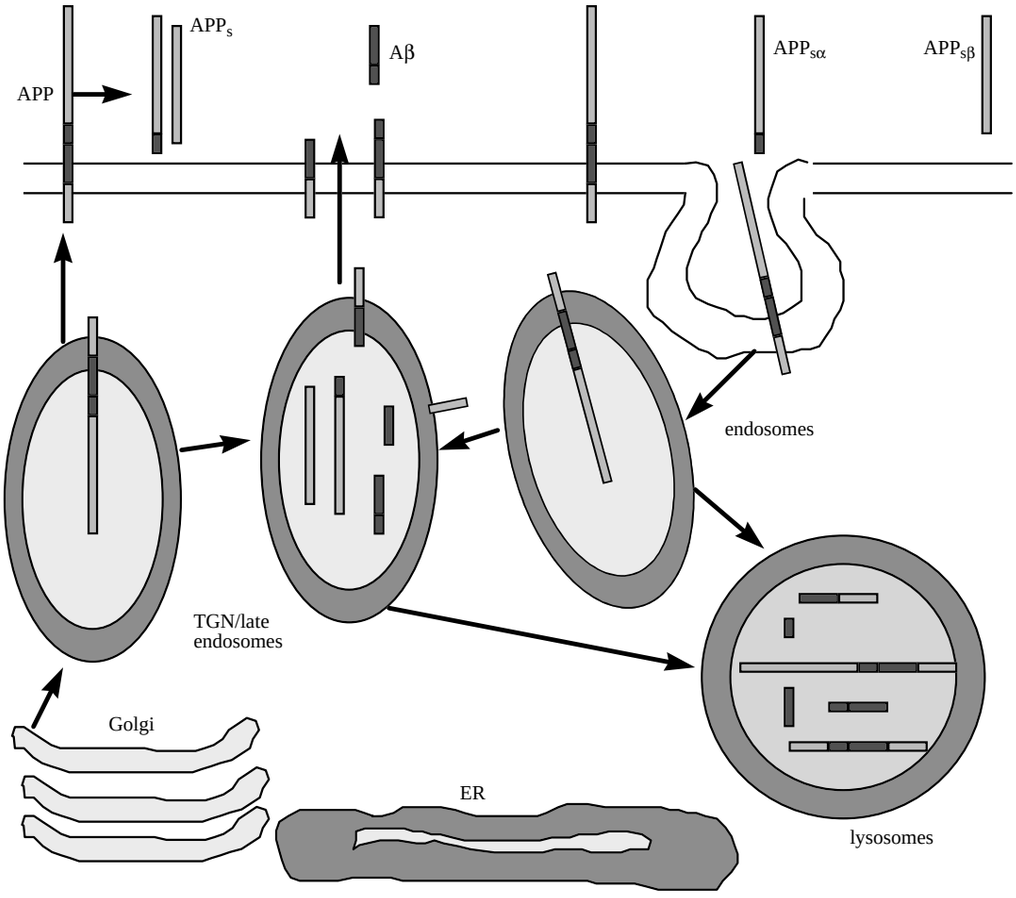

Figure 4. The trafficking and sites of processing of APP. APP is synthesized in the endoplasmic reticulum (ER) and passes through the Golgi apparatus to the trans-Golgi/late endosome compartments. Some APP is processed at the cell surface and other molecules are reinternalized after which they may be recycled to the cell surface or proteolysed and the products secreted; other APP molecules are probably completely degraded in the lysosomes.

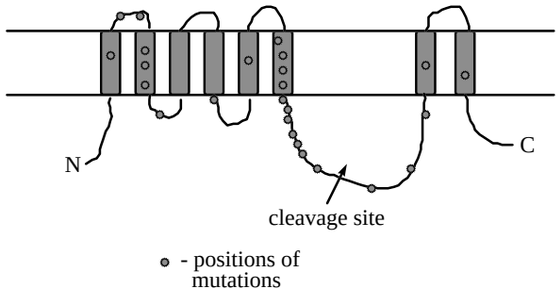

Figure 5. Presenilin structure. Presenilins are mainly located in internal membranes and the polypeptide traverses the membrane eight times. The approximate locations of sites of some of the known mutations are shown.

two-dimensional gel analysis (Sergeant *et al.* 1995) as well as SDS-PAGE (SDS-polyacrylamide gel electrophoresis) (B.H.A., G. Gibb, D. Davis and D. P. Hanger, unpublished observations), PHF-tau appears to be more heavily phosphorylated than the hyperphosphorylated fractions of foetal and normal adult tau. Thus, PHF-tau may be phosphorylated at sites additional to those in non-pathological forms of tau, for which there is some evidence (Shiurba *et al.* 1996; Hasegawa *et al.* 1996), or PHF-tau may be 'frozen' in a hyperphosphorylated state. Evidence in favour of the latter is demonstrated by the rapid increase in hyperphosphorylation of cellular tau induced by phosphatase inhibitors (Davis *et al.* 1995; Arendt *et al.* 1995), suggesting that phosphate on tau is turning over rapidly and that a proportion of normal tau is always hyperphosphorylated. Thus, PHF-tau may somehow become 'frozen' in a hyperphosphorylated state with phosphatases no longer able to remove phosphate, possibly because of aggregation of the tau into PHF which might be triggered by generation of a truly abnormally phosphorylated site or by factors such as proteoglycans (Goedert *et al.* 1996).

It is likely that the developmental regulation of tau phosphorylation is important because of the profound effect this has on microtubule dynamics, and it has been shown that the phosphorylation of tau by GSK-3 both *in vitro* and in cells affects microtubule properties. *In vitro*, tau promotes nucleation of microtubules, resulting in a population of shorter but more abundant microtubules and phosphorylation by GSK-3 abolishes this property of tau, which mutagenesis studies have

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shown resides in several phosphorylation sites (Utton *et al.* 1997). In transfected non-neuronal cells, tau bundles microtubules and induces neurite-like extensions (Kanai *et al.* 1992), but hyperphosphorylation of tau by GSK-3 in co-transfected COS cells abolished the microtubule-bundling properties of tau with a concomitant reduction in microtubule stability (Lovestone *et al.* 1996*b*). Thus, phosphorylation of tau by GSK-3 clearly results in regulation of its microtubule-modulating properties and leads to the conclusion that increased hyperphosphorylation or induction of a 'frozen' hyperphosphorylated state may be a factor in the observed loss of assembled microtubules in PHF-afflicted neurones, accounting for the cytoskeleton-PHF transition (figure 6).

(*e*) *Apolipoprotein E*

There have been a number of attempts to explain the genetic risk of possessing an apolipoprotein ε4 allele but so far none have resulted in convincing mechanisms. Apolipoprotein E4 protein has been claimed to promote aggregation of Aβ more strongly than apolipoprotein E3 (Strittmatter *et al.* 1993), but other studies have found the opposite effect and this may depend upon the source of apolipoprotein E used for the experiments (LaDu *et al.* 1994). Similarly, it has been proposed that apolipoprotein E3 binds tau more strongly than apolipoprotein E4 and may therefore protect tau from hyperphosphorylation (Fleming *et al.* 1996). This is a somewhat surprising proposal because apolipoproteins are normally not expected to gain access to the cytoplasmic compartment of cells where tau is present. However, there are studies that show the presence of apolipoprotein E in the cytoplasm and our laboratory has reported that tau may have a role in the selective retention of apolipoprotein E3 over E4 in the cytoplasm (Lovestone *et al.* 1996*a*). More studies are required to elucidate the mechanism by which the apolipoprotein allotype influences the pathogenesis of Alzheimer's disease and in view of the recently reported combined association of herpes simplex virus and apolipoprotein ε4 alleles accounting for increased risk of Alzheimer's disease, it may be that apolipoprotein E influences neuronal susceptibility to herpes infection (Itzhaki *et al.* 1997).

(*f*) *The amyloid cascade*

The amyloid cascade hypothesis (figure 7) proposes that Aβ deposition in the brain is the trigger for the rest of the pathogenesis of Alzheimer's disease, including PHF formation (Hardy & Higgins 1992). Although there is now good experimental evidence in support of altered APP metabolism leading to senile plaques, there is little evidence in support of this also giving rise to PHF. Two groups have claimed that treatment of cultured neurones with Aβ leads to increased tau phosphorylation (Busciglio *et al.* 1995; Takashima *et al.* 1996), although work in our laboratory has failed to confirm such observations (Davis *et al.* 1995). It may be time, therefore, to look for alternative links between APP/Aβ and tau hyperphosphorylation/PHF formation.

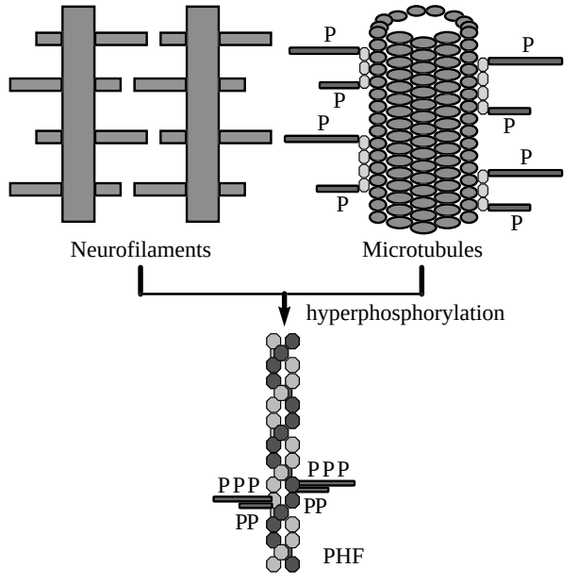

Figure 6. The cytoskeleton to PHF transition. The normal cytoskeleton in neurones consists principally of microtubules and neurofilaments. When PHF form, the cytoskeleton disappears and is replaced by PHF containing hyperphosphorylated tau.

Since phosphorylation of proteins is regulated by signal transduction events and it has been demonstrated by many laboratories that APP metabolism is modulated by activation of protein kinase C (PKC) (Efthimiopoulos *et al.* 1994; Nitsch *et al.* 1996; Govoni *et al.* 1996*a*), it may be timely to investigate the possible links between APP metabolism and tau phosphorylation that are mediated by signal transduction processes. There have been reports that PKC activity is depressed in Alzheimer brain and fibroblasts (Van-Huynh *et al.* 1989; Govoni *et al.* 1996*a*,*b*). A reduction in PKC activity would be compatible with increased amyloidogenic processing of APP and it has been reported that PKC activation reduces GSK-3β activity (Cook *et al.* 1996), thus again a reduction in PKC might lead to tau hyperphosphorylation. Future work may, therefore, be directed towards investigating if such a coupling between APP metabolism and tau phosphorylation via PKC occurs in neurones and *in vivo*.

5. OTHER RELATED NEURODEGENERATIVE DISEASES

Alzheimer's disease is the commonest neurodegenerative disease but other less common diseases may be related since several are characterized by neuronal inclusion bodies that are composed of cytoskeletal proteins, including tau (table 2). Since Alzheimer's disease possibly represents a form of accelerated or accentuated ageing, then these other conditions might also be similarly considered. There is evidence of Lewy bodies in the substantia nigra in putative presymptomatic Parkinson's disease (Gibb & Lees 1988).

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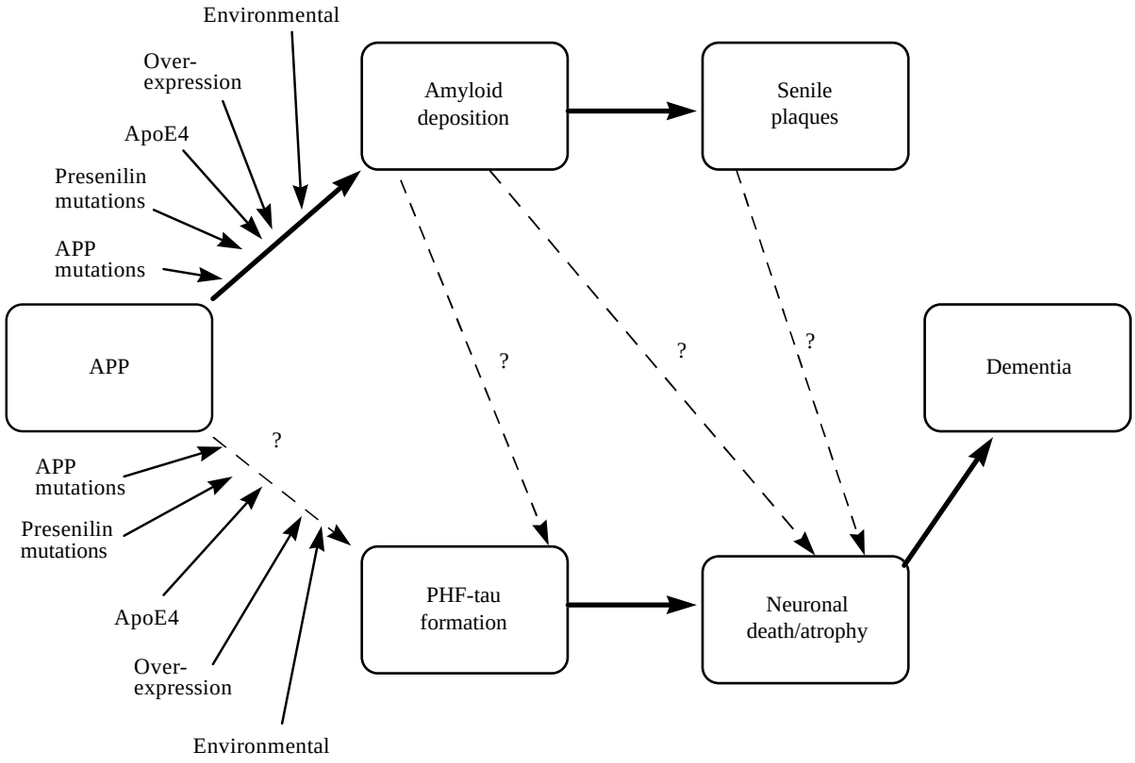

Figure 7. The amyloid cascade hypothesis. APP is presumed to be involved at an early stage and dementia is the result of neuronal loss or atrophy. Solid lines represent stages for which there is good experimental evidence whereas dotted lines indicate possible but unknown relationships.

Table 2. *Neurodegenerative diseases—inclusion bodies and cytoskeletal constituents*

neurodegenerative disease	inclusion body	ultrastructural component	main constituent cytoskeletal protein
Alzheimer's disease	neurofibrillary tangles	PHF and some straight filaments	tau
dementia with Lewy bodies	cortical Lewy bodies	neurofilaments	neurofilament triplet proteins
Parkinson's disease	Lewy bodies	neurofilaments	neurofilament triplet proteins
motor neurone disease	axonal spheroids and perikaryal inclusions	neurofilaments	neurofilament triplet proteins
Pick's disease	Pick bodies	straight filaments	tau
progressive supranuclear palsy (PSP)	PSP neurofibrillary tangles	straight filaments	tau
multiple system atrophy (MSA)	glial cytoplasmic inclusions	tubules	tau
corticobasal degeneration	neuronal inclusions	straight filaments	tau
Huntington's disease	none		

Dementia is also the consequence of neurodegeneration caused by some of these other rarer diseases such as multiple system atrophy or progressive supranuclear palsy, both of which have inclusions composed of tau (Jellinger 1996). Thus, with advancing age there may be an increasing risk of neurodegeneration and depending upon the interaction of genotype with environmental factors, one or other of these diseases of the nervous system may ensue.

I wish to thank Dr Rob Howard for providing the MRI scans for figure 1. I also thank the Wellcome Trust and the Medical Research Council for financial support for some of the research referred to in this article.

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