Pathological changes leading to neuronal degeneration in Alzheimer's disease

by

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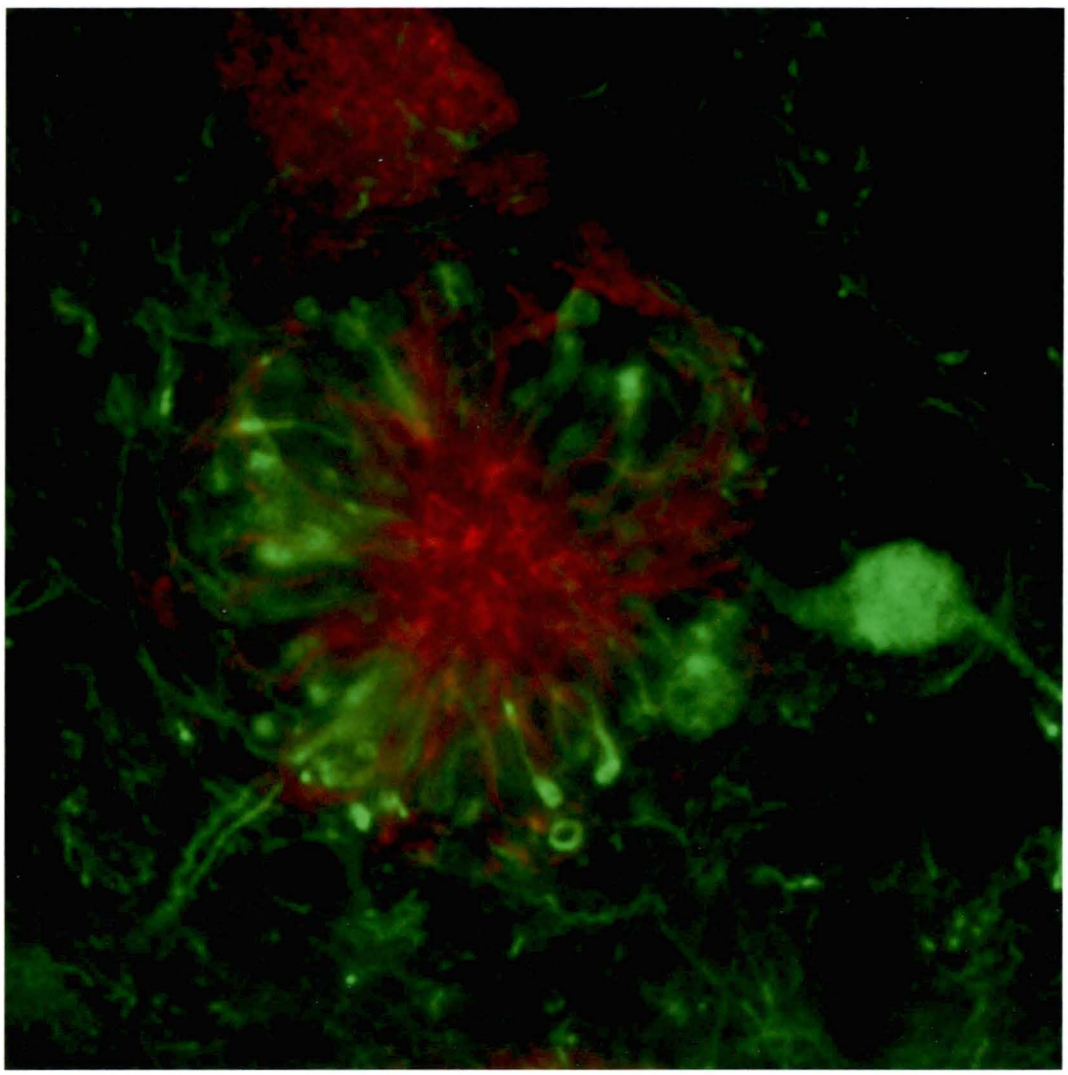
A β-amyloid-labelled (red) plaque associated with abundant α-internexin-labelled dystrophic neurites (green) in a 15 month old transgenic Tg2576 Alzheimer's disease mouse.



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SUMMARY

Alzheimer's disease (AD) is the most common form of dementia. The pathological hallmarks of AD include β-amyloid (Aβ) plaques, dystrophic neurites (DNs) and neurofibrillary tangles (NFTs). All of these pathological hallmarks involve abnormal insoluble proteinaceous aggregates that have the capacity to disturb normal cellular functioning. However, there is disagreement within the AD literature as to whether it is Aβ plaques, soluble Aβ or NFTs that are the primary causative agent of AD. Irrespective of the initial cause of AD, the burden of Aβ plaques and NFTs increases as AD progresses, eventually resulting in substantial brain atrophy, which is at least partially due to overt neuronal degeneration and death. Thus, a better understanding of the aetiology and progression of AD will enable more efficient therapeutics to be developed.

This thesis investigates the role of apoptosis in AD with the aim of ascertaining whether apoptosis is involved in disease staging or progression or is a causative agent of AD. No increase in immunohistochemical labelling, or change in localisation that distinguished between control, preclinical AD and AD cases were present for a range of apoptosis-related proteins. In addition, mRNA levels of apoptosis-related proteins differed little between control, preclinical AD and AD cases when analysed by real time reverse transcriptase polymerase chain reaction. There was no difference in the percentage of apoptotic-like nuclei in the neocortex between control, preclinical AD and AD cases and very few of the nuclei associated with Aβ plaques or NFTs were abnormal. Cytochrome c (cyto c)-labelling was punctate in cortical neurons, including a subset of NFT-bearing neurons, but a subset of DNs demonstrated cytoplasmic cyto c-labelling. These data suggest that apoptosis may not play a major role in the pathogenesis or progression of AD and that activation of apoptotic pathways can occur in the absence of extensive terminal apoptosis in the brain.

As post-mortem human AD tissue provides only random time points in the dynamic process of disease progression, transgenic mouse models of AD are invaluable tools for investigating aspects of age-associated disease progression and also for testing potential therapeutics for AD. Although, for animal models to be used effectively, a detailed understanding of the pathology and disease processes that they model is required. Therefore, the Aβ plaque-associated neuronal pathology in two transgenic AD mouse models was investigated to determine whether the neuronal pathology in these mice more closely resembles human preclinical AD cases or clinically evident AD cases. Using immunohistochemistry the morphology and neurochemistry of the Aβ plaque-associated DNs present in the two lines of transgenic AD mice was demonstrated to be strikingly similar to that in human preclinical AD cases, but not AD cases. Importantly, quantitation demonstrated that the Aβ plaques in these transgenic AD mice were highly axonopathic, and were also associated with displaced or clipped apical dendrites in both transgenic mouse models. The results suggest that these mice represent an accurate and valuable model of preclinical AD that can be utilised as a platform for testing potential therapeutic agents for AD, to be administered prior to extensive neuronal loss.

Finally, as current treatments for AD only treat the symptoms of the disease and do not slow or stop its progression, the potential of a novel therapeutic agent with zinc binding, neuroprotective and anti-oxidant properties, metallothionein isoform IIA (MTIIA), was investigated. Utilising immunohistochemistry, the Aβ and thioflavine s plaque loads and the Aβ plaque-associated neuronal pathology in 13 and 15 month old Tg2576 mice was investigated after three months of metallothionein IIA treatment. Although this pilot study did not produce any statistically significant results, there was a trend towards lower Aβ and thioflavine s plaque loads in MTIIA treated Tg2576 mice. Thus, MTIIA warrants further investigation as a potential therapeutic for AD in the future.

The research in this thesis provides valuable new data on the staging of AD, with particular regard to the role of apoptosis in AD and Aβ plaque-associated neuronal pathology in transgenic AD mice and human AD. The current study indicates that

apoptosis does not play a seminal role in the genesis or progression of AD pathology. This works has also clarified how two widely studied transgenic AD mice compare to the schema of disease progression that occurs in human AD, and strongly suggests that these transgenic AD mice mimic human preclinical AD. Finally, a pilot study of MTIIA administration to transgenic AD mice did not result in significant differences between the treatment and control groups.

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ABBREVIATIONS

Active caspase-3 (aC3)

Active caspase-8 (aC8)

Active caspase-9 (aC9)

Apolipoprotein E (ApoE)

Alzheimer's disease (AD)

β-amyloid (Aβ)

β-amyloid precursor protein (APP)

Blood brain barrier (BBB)

Brain derived neurotrophic factor (BDNF)

Cornu ammonis (CA)

Central nervous system (CNS)

Chromogranin A (CgA)

Cyclin dependent kinase 5 (cdk5)

Cytochrome c (cyto c)

Deoxyribose nucleic acid (DNA)

Docosahexaenoic acid (DHA)

Dystrophic neurites (DNs)

Fas associated death domain (FADD)

Growth associated protein 43 (GAP43)

Glial fibrillary acidic protein (GFAP)

Glycogen synthase kinase 3β (GSK3β)

Inferior temporal gyrus (ITG)

Metallothionein (MT)

Metallothionein isoforms I and II (MTI/II)

Microtubule associated protein (MAP)

Nerve growth factor (NGF)

Neurofibrillary tangles (NFTs)

Neurofilament (NF)

Neurofilament triplet protein heavy (NFH)

Neurofilament triplet protein light (NFL)

Neurofilament triplet protein medium (NFM)

Nonsteroidal anti-inflammatory medications (NSAIDs)

N-methyl-D-aspartate (NMDA)

Paired helical filaments (PHFs)

Phosphate buffered saline (PBS)

Ribonucleic acid (RNA)

Reverse transcriptase polymerase chain reaction (RT-PCR)

Room temperature (RT)

SEM (standard error of the mean)

Superior frontal gyrus (SFG)

TNF receptor-associated death domain (TRADD)

Tumour necrosis factor receptor (TNFR)

Western blotting (WB)

1 INTRODUCTION

1.1 THE PATHOLOGICAL HALLMARKS OF AD

Alzheimer's disease (AD) is the most common neurodegenerative disease (Bossy-Wetzel et al., 2004) and is likely to become an increasing problem as the world's population ages (Vickers et al., 2000). The main pathological characteristics of AD are β–amyloid (Aβ) plaques, dystrophic neurites (DNs), neurofibrillary tangles (NFTs) and neuropil threads (Braak and Braak, 1991). All of these pathological hallmarks consist of abnormal accumulations of proteins within the brain. Plaques are comprised of Aβ protein that undergoes an abnormal conformational change to form insoluble fibrils that aggregate into extracellular masses. β-amyloid protein is derived from the β-amyloid precursor protein (APP) and is normally synthesised and secreted by neuronal and non-neuronal cells (Busciglio et al., 1993; Fraser et al., 1993; Haass and Selkoe, 1993; Martin et al., 1994; Hartmann et al., 1997; Mesulam, 1999). There are two main processing pathways for APP (Reid et al., 2007). The pathway that results in the production of Aβ begins with the cleavage of APP by β-secretase, which is usually the β-site APP-cleaving transmembrane aspartic protease 1 (BACE1), to produce APPsβ, the remaining C-terminal fragment is then cleaved by the γ-secretase complex to produce Aβ and the APP intracellular domain (Zheng and Koo, 2006; Reid et al., 2007). The γ-secretase complex consists of presenilin, nicastrin, anterior pharynx-defective-1 and presenilin-enhancer-2 in a stable complex (DeStrooper, 2003) and can cleave APP at heterogenous sites between 38-43 amino acids to produce the numerous Aβ peptides (Reid et al., 2007). In the second cleavage pathway APP is cleaved by α-secretase, one of several members of the disintegrin and metalloprotease enzyme family, within the Aβ domain to produce APPsα, and the C-terminal fragment is then cleaved by the γ-secretase complex to yield a 3kDa product and the APP intracellular domain (Zheng and Koo, 2006; Reid et al., 2007). β-amyloid plaques are present within the brains of AD patients and mildly demented elderly (Iwatsubo et al., 1994; Morris et al., 1996; Braak and Braak, 1997; Price and Morris, 1999) and can be morphologically characterised into

three varieties: diffuse, fibrillar and dense cored (Dickson et al., 1988; Armstrong, 1998; Dickson and Vickers, 2001). In contrast to Aβ plaques, DNs, NFTs and neuropil threads all involve abnormal intraneuronal filamentous (neurofibrillary) accumulations of altered cytoskeletal proteins. Dystrophic neurites are aberrantly shaped neuronal processes, likely of axonal origin, with aberrant accumulations of cytoskeletal proteins and cytoskeletal-associated proteins, which are specifically associated with Aβ plaques. However, not all Aβ plaques are associated with DNs, and the subset of Aβ plaques that are associated with DNs are referred to as neuritic Aβ plaques. Neurofibrillary tangles are intraperikaryal inclusions, principally comprised of altered tau proteins that occur within specific subsets of neurons in the cerebral cortex and selected subcortical nuclei. Similarly, neuropil threads are also accumulations of abnormal tau proteins that principally occur in the dendrites of NFT-bearing nerve cells (Braak and Braak, 1988; Braak et al., 1996).

1.1.1 Controversial pathogenic role of Aβ in AD

The 'amyloid cascade hypothesis' asserts that the primary cause of AD is Aβ protein. In this regard, it is proposed that Aβ plaques may be toxic to nerve cells and their processes, which is supported by *in vitro* studies that demonstrate that aggregated Aβ is particularly toxic to neurons (Pike et al., 1993; Lorenzo and Yanker, 1994; Ivins et al., 1998; Lambert et al., 1998; Hartley et al., 1999). However, other proponents of the Aβ cascade hypothesis state that it is soluble Aβ oligomers or protofibrils that are toxic to neurons and ultimately result in neurodegeneration (Lambert et al., 1998; Hartley et al., 1999; Wang et al., 2002; Lanz et al., 2003). Recent research suggests that oligomeric Aβ in particular, affects synaptic transmission (Huang et al., 2006; Lacor et al., 2007; Matsuyama et al., 2007). It is also possible that both soluble and aggregated fibrillar Aβ have neurotoxic properties.

Alternatively, there is the opposing view that Aβ is not the primary causative agent of AD (Martin et al., 1994; Terry, 1996; Joseph et al., 2001). In this respect, neurofibrillary changes are emphasised as having a more central pathological role in

AD. Thus, there is disagreement as to which pathological hallmark of AD is best linked to the dementia phenotype. While the load of neuritic Aβ plaques in an individual has been reported to be the best indicator of the degree of dementia (Cummings and Cotman, 1995) others have shown that NFTs (McKee et al., 1991; Arriagada et al., 1992; Bierer et al., 1995; Mesulam, 1999; Gold et al., 2001; Giannakopoulos et al., 2003), DNs (McKee et al., 1991) the density of synaptophysin-labelled synapses (Terry et al., 1991) or neuron loss (Bussière et al., 2003; Giannakopoulos et al., 2003) more reliably correlate with cognitive deterioration in AD. However, it is possible that neuritic Aβ plaques, NFTs and synaptic and neuronal cell loss all contribute to the cognitive decline in AD. In addition, the temporal and spatial pattern of Aβ plaque deposition is different to that of NFTs (Arriagada et al., 1992; Lue et al., 1996; Knowles et al., 1998; Price et al., 1999) making it difficult to ascertain whether one pathology causes or is linked to the other.

Genetic studies, the production of transgenic APP mice and the analysis of human brain material all provide general support for the amyloid cascade hypothesis. For example, APP, presenilin 1 (PS1) and presenilin 2 (PS2) gene mutations have been linked to familial forms of AD and it has been proposed that all of these gene defects ultimately affect the metabolism of APP (Beyreuther and Masters, 1997; Hardy et al., 1998), although the precise role of the PS1 and PS2 gene products in AD pathology is controversial. 'Normal' APP processing results in a heterogeneous mixture of proteins, including Aβ (Haas and Selkoe, 1993). However, the presence of such AD-causing mutations results in the production of proportionally more of the hydrophobic Aβ protein that is likely to precipitate insoluble Aβ plaques (for review see Sandbrink and Beyreutheris, 1996; Selkoe, 1996; Bossy-Wetzel et al., 2004). Further genetic support for the Aβ cascade hypothesis comes from the study of Downs syndrome. Individuals with Downs syndrome have an extra copy of the APP gene and as a result develop Aβ plaques at a very early age, followed by other neuropathological features of AD (Brion, 1996; Lemere et al., 1996). This implies a staging of AD, with APP abnormalities and Aβ plaque formation preceding, and possibly causing, neurofibrillary degeneration. Furthermore, numerous transgenic mouse models of AD expressing a variety of

combinations of human APP, PS1 and PS2 proteins harbouring mutations associated with familial AD exhibit AD-associated pathology with aging, including Aβ plaques, DNs, astrogliosis, microgliosis and neurodegeneration, however, these mice do not demonstrate neurofibrillary pathology or extensive neuronal death (Games et al., 1995; Hsiao et al., 1996; Borchelt et al., 1997; Sturchler-Pierrat et al., 1997; Holcomb et al., 1998; Moechars et al., 1999; Janus et al., 2000; Mucke et al., 2000; Chishti et al., 2001; Blanchard et al., 2003; Higgins and Jacobsen, 2003; Richards et al., 2003; Cheng et al., 2004; Kawasumi et al., 2004; Oakley et al., 2006).

Despite this wealth of information on genetically linked AD, it is important to note that the majority of AD cases are sporadic, with genetically linked forms accounting for less than 5% of all AD cases (van Leeuwen et al., 1998), however, genetically linked and sporadic AD cases may share a common final pathophysiological pathway (Lippa et al., 1996). For example, when two genetically linked AD cases were compared to sporadic cases there was no difference in the pattern of distribution of neuronal loss, Aβ plaques, neuritic Aβ plaques and NFTs, or in the ratio of neuronal loss to neuritic Aβ plaques or NFTs (Lippa et al., 1996). However, a recent study utilising *in vivo* imaging techniques suggests that human subjects carrying PS1 mutations that lead to early-onset AD demonstrate a different regional pattern of Aβ plaque deposition to that in sporadic cases (Klunk et al., 2007). It has been suggested that sporadic AD cases begin with an increase in production of Aβ or a decrease in the clearance of Aβ, particularly with regard to the hydrophobic Aβ protein (Selkoe et al., 1996), resulting in the aggregation of Aβ into insoluble Aβ plaques and the onset of degeneration. In support of this proposition, the activity of the APP cleaving enzyme β-secretase significantly increases with aging (Fukumoto et al., 2004), while the ε4 allele of apoplipoprotein E (ApoE), which is a major risk factor for sporadic AD, promotes Aβ precipitation into insoluble Aβ plaques (Mesulam, 1999).

It has been suggested that low levels of free testosterone may also be a risk factor for developing AD, with low levels of testosterone correlating with cognitive dysfunction,

including in subjects with mild cognitive impairment and AD (Hogervorst et al., 2004; Beauchet, 2006). Furthermore, gonadectomized male 3xTg-AD mice exhibited increased accumulation of Aβ and deficits in hippocampal function that were attenuated by dihydrotestosterone treatment (Rosario et al., 2006). However, other population studies have suggested that there was no association between oestrogen or testosterone levels in men and the risk of developing AD (Ravaglia et al., 2007). In comparison, the role of oestrogen and progesterone as risk factors for women developing AD and as factors that influence the pathogenesis of AD are controversial. Some studies suggest that oestrogen and progesterone are neuroprotective and decrease the risk of AD (Yue et al., 2005; Carroll et al., 2007), while other investigations indicate that increased exposure to oestrogen and progesterone increase the risk of developing AD in women (Shumaker et al., 2003; Shumaker et al., 2004; Colucci et al., 2006; Ravaglia et al., 2007).

1.1.2 A "mass effect" variant of the amyloid cascade hypothesis

Following findings that indicate that DNs, particularly in the earliest stages of AD, exhibit morphological and biochemical features that are strikingly similar to the axonal response to structural injury (Masliah et al., 1993; Meller et al., 1994; DeWitt and Silver, 1996; Su et al., 1996a; Vickers et al., 1996; Christman et al., 1997; King et al., 1997; Nakamura et al., 1997; Dickson et al., 2000; King et al., 2001; Chuckowree and Vickers, 2003; Dickson et al., 2005) a variant of the amyloid cascade hypothesis was proposed (reviewed in Vickers, 1997; King et al., 2000, Vickers et al., 2000). Thus, it is hypothesised that Aβ plaque formation, particularly the more dense Aβ plaques, causes physical deformation and injury to axons, resulting in a cascade of cytoskeletal changes that lead to DN formation (King et al., 1997; Vickers, 1997; Dickson et al., 1999, Vickers et al., 2000; Dickson et al., 2005). Indeed, only non-neuritic Aβ plaques are present in young transgenic AD mice, while Aβ plaque-associated DNs do not appear until several months later (Mucke et al., 2000). Additionally, fewer dendrites, axons and neuronal somata are present within the areas inhabited by Aβ plaques in human AD (DeWitt and Silver, 1996; Knowles et al., 1998; Tomikodoro et al., 2001; Adlard and Vickers, 2002), supporting the proposal that Aβ plaques can be considered as space-

consuming entities that push other structures aside and/or compress neural elements. Thus, individual Aβ plaques may create a localised "mass effect" within the neuropil.

However, experimental models of injury generally involve acute axonal injury, after which the damaged axons undergo reactive and regenerative changes and the injury is eventually resolved. As dense Aβ plaques are considered to be relatively stable entities (Christie et al., 2001; Spires et al., 2005), the physical trauma inflicted upon neurites in AD is chronic and may interfere with the "normal" response to axonal injury. Thus, it is postulated that chronic stimulation of the neuronal reaction to injury by Aβ plaques, perhaps over many years, may cause changes in cytoskeletal elements such as neurofilaments (NFs) and tau that lead to the formation of the abnormal insoluble filamentous structures, such as those in NFTs (Vickers et al., 1996; Dickson et al., 1999; Metsaars et al., 2003). To elaborate on the data leading to the formation of this mass effect hypothesis, the following sections of this review focus on the early neuronal changes that are specifically associated with Aβ plaque pathology, and how the staging of neuronal alterations provides clues to the pattern of neural degeneration that underlies dementia.

1.2 CYTOSKELETAL PATHOLOGY IN AD

Neurofibrillary pathology involves specific abnormal alterations in neuronal cytoskeletal proteins. The cytoskeleton is a rigid yet dynamic framework of protein polymers that determines neuronal morphology and intracellular structure and also plays an important role in axonal transport. The cytoskeleton is made up of three classes of protein polymers; the microtubules, NFs and microfilaments. The NFs are intermediate neuronal filaments that include specific protein classes such as the NF triplet proteins, peripherin and α-internexin. The NF triplet proteins include NFL, NFM and NFH (low, medium and high molecular weight subunits), which normally co-assemble to form intermediate filaments (Smith et al., 2003). Recent research shows that α-internexin also acts as a fourth subunit that co-assembles with the NF triplet proteins in the mature central nervous system (CNS) (Yuan et al., 2006). Neurofilament triplet proteins and α-

internexin are present in specific subsets of neurons, with NF triplet proteins being richly expressed in large neurons especially those with long axonal processes (Su et al., 1996a; Kirkcaldie et al., 2002; Dickson et al., 2005). In contrast, α-internexin is more widely distributed amongst cortical cell types (Dickson et al., 2005) and is present in a substantial proportion of human and rat neocortical cells that are not labelled for the NF triplet proteins. As discussed below, alterations in NF proteins, particularly in DNs, may provide important insight into the deleterious effects of Aβ plaque formation on the neuropil. In addition, there is on-going and substantial interest in the role of the microtubule-associated protein, tau, in neuronal degeneration in AD. The normal biological role of tau is to promote the assembly of tubulin into microtubules and aid microtubule stability, and this is regulated by the phosphorylation state of tau (Trojanowski and Lee, 1994; Hardy et al., 1998; Iqbal et al., 2005). Abnormal hyperphosphorylation of tau results in the loss of normal tau function, increases the ability of tau to aggregate with itself and other microtubule associated proteins in insoluble aggregates, and precedes the accumulation of tau into insoluble masses in AD (as reviewed in Iqbal et al., 2005). Abnormal accumulations of tau are found in DNs, NFTs and neuropil threads (Trojanowski and Lee, 1994; Johnson and Jenkins, 1999). Neurofibrillary tangles can be classified according to their sequential changes in morphology (Braak et al., 1994). Group one or pre-NFT neurons do not exhibit established NFTs, but do contain paired helical filament (PHF) tau (Braak et al., 1994). Group two and three neurons progressively accumulate intracellular NFTs, and group four and five NFTs are present in the extracellular space following the death of the NFT-bearing neurons (Braak et al., 1994). However, the abnormally phosphorylated form of tau that NFTs and neuropil threads are principally comprised of is different to the form of tau that is present in DNs (Onorato et al., 1989; Trojanowski and Lee, 1994; Johnson and Jenkins, 1999).

1.3 DYSTROPHIC NEURITE PATHOLOGY IN AD

Dystrophic neurites are aberrant neuronal processes that can be immunoreactive for both phosphorylated and dephosphorylated NF triplet proteins, APP, ubiquitin, α-internexin

and/or tau (Dickson et al., 1988; Cras et al., 1991; Benzing et al., 1993; Masliah et al., 1993; Su et al., 1996a; Vickers et al., 1996; King et al., 1997; Nakamura et al., 1997; Su et al., 1998; Thal et al., 1998; Dickson et al., 1999; Dickson and Vickers, 2001; Dickson et al., 2005). Morphologically, DNs appear as swollen tortuous neurites 10-60μm in diameter with a range of shapes that are invariably associated with Aβ plaques (Benzing et al., 1993; Masliah et al., 1993; Su et al., 1996a; Vickers et al., 1996; King et al., 1997; Su et al., 1998; Dickson et al., 1999; Dickson and Vickers, 2001; Dickson et al., 2005). Dystrophic neurites immunolabelled for NF triplet proteins and α-internexin can be categorised as bulb- or ring-like structures based on the morphology of labelled elements (Masliah et al., 1993; Su et al., 1996a; Vickers et al., 1996; Su et al., 1998; Dickson et al., 1999; Dickson and Vickers, 2001). Bulb-like DNs appear as bulbar swellings whereas ring-like DNs are generally smaller and appear as spherical structures with a hollow core (Dickson et al., 1999; Dickson and Vickers, 2001). Both ring- and bulb-like DNs may either be continuous with an axon or appear as isolated structures (Dickson et al., 1999; Dickson and Vickers, 2001). The morphological and biochemical properties of DNs differ between the early and late stages of AD (Benzing et al., 1993; Masliah et al., 1993; Su et al., 1996a; Vickers et al., 1996; Su et al., 1998; Dickson et al., 1999; Dickson and Vickers, 2001; Dickson et al., 2005).

1.3.1 Characteristics of DNs in preclinical AD

Defining the key cellular processes involved in AD has been difficult due to the complex brain pathology of this degenerative condition (Benzing et al., 1993). However, cases with mild dementia exhibit significantly more NFTs and neuritic Aβ plaques than non-demented elderly brains (Morris et al., 1996; Price and Morris, 1999). As there is strong evidence that AD begins many years before the clinical symptoms are evident, a 'pathologically aged' or 'preclinical phase' of AD has been defined in which the initial signs of the pathological hallmarks of AD are present in the cortices typically associated with minor cognitive deficits that may represent incipient AD dementia (Morris et al., 1996; Price and Morris, 1999; Vickers et al., 2000). This 'pathological aging' is characterised by the emergence of widespread extracellular Aβ plaques within the

cerebral cortex but no overt nerve cell degeneration or neurofibrillary changes. Thus, non-demented or mildly demented elderly brains with numerous Aβ plaques are studied as a potential preclinical stage of AD, and may provide important clues to the initiation of neuronal pathology (Benzing et al., 1993; Lue et al., 1996; Vickers et al., 1996; Dickson et al., 1999; Dickson and Vickers, 2001). In preclinical AD cases Aβ plaque-associated DNs are frequently labelled with antibodies to APP, NF triplet proteins and α-internexin, but not for the abnormal tau protein that characterises neurofibrillary pathology in established and end-stage AD (Cras et al., 1991; Benzing et al., 1993; Vickers et al., 1996; Su et al., 1998; Dickson et al., 1999; Dickson and Vickers, 2001; Dickson et al., 2005). The DNs in preclinical AD cases include ring- and bulb-like structures, which are labelled for both α-internexin and NF triplet proteins (Dickson et al., 1999; Dickson et al., 2005).

1.3.2 Characteristics of DNs in AD

In contrast, tau-labelled DNs in AD cases are abundant and typically appear as angular, elongated structures (Dickson et al., 1999). Interestingly, NF triplet protein and α-internexin-labelled bulbous and 'sprouting' DNs are also abundant, but NF triplet protein-labelled ring-like dystrophic neurites are rare, while α-internexin-labelled ring-like DNs are numerous (Dickson et al., 1999; Dickson et al., 2005). A substantial subset of the NF triplet protein and α-internexin DNs in AD cases exhibit a core of tau immunoreactivity (Su et al., 1998; Dickson et al., 1999; Dickson et al., 2005). This suggests that DNs may "mature" from NF triplet protein and α-internexin immunoreactive DNs into DNs that label for tau only (Su et al., 1998; Dickson et al., 1999; Dickson et al., 2005). In support of this proposition, two different AD mouse models exhibit DNs that are immunoreactive for phosphorylated NF triplet protein epitopes several months before phosphorylated tau-labelled DNs appear (Masliah et al., 2001; Blanchard et al., 2003). Thus, DNs may begin as the NF triplet protein- and α-internexin-immunoreactive DNs found in preclinical AD and then develop a core of altered tau filaments (Su et al., 1998; Dickson et al., 1999; Dickson et al., 2005). Finally, the tau-immunolabelled elements within the DN may develop and expand,

eventually replacing all normal cytoskeletal proteins in these axonal segments (Su et al., 1998; Dickson et al., 1999; Dickson et al., 2005).

1.3.3 Morphological and biochemical changes in DNs are identical to reactive axonal alterations following axonal injury

Many *in vivo* and *in vitro* models of axonal injury produce reactive axonal structures that are neurochemically and morphologically similar to the neurofilamentous DN pathology in AD. Both phosphorylated and dephosphorylated NF triplet proteins and α-internexin-labelled ring- and bulb-like structures similar to those in AD brains are observed following axonal injury (Masliah et al., 1993; Meller et al., 1994; DeWitt and Silver, 1996; Su et al., 1996a; Vickers et al., 1996; Christman et al., 1997; King et al., 1997; Nakamura et al., 1997; Dickson et al., 2000; King et al., 2001; Chuckowree and Vickers, 2003; Dickson et al., 2005; Table 1.1). The presence of dephosphorylated NF triplet protein in DNs is particularly interesting as these proteins are normally located in the somatodendritic neuronal domain (Rosenfeld et al., 1987), but are abnormally present in axons following physical trauma (Meller et al., 1994; King et al., 1997; Dickson et al., 1999; King et al., 2001).

Previous studies have demonstrated that ring- and bulb–like structures immunolabelled for NF triplet protein and/or α-internexin represent a stereotypical reactive response of axons to damage in both a model of structural injury to adult rat neocortex as well as

Table 1.1 The morphology and neurochemistry of DNs in AD are strikingly similar to the to the reactive axonal alterations that occur following axonal injury.

	Neurochemical profile of DNs in AD	**Neurochemical profile of injured axons**	
		In vitro	***In vivo***
NF triplet proteins	Benzing et al., 1993 Masliah et al., 1993 Su et al., 1996a King et al., 1997 Su et al., 1998 Nakamura et al., 1999 Dickson et al., 1999 Dickson and Vickers, 2001 Dickson et al., 2005	Dickson et al., 2000 Chuckowree and Vickers, 2003 Dickson et al., 2005	Meller et al., 1994 King et al., 1997 King et al., 2001 Dickson et al., 2005
α-internexin	Dickson et al., 2005	Dickson et al., 2005	Dickson et al., 2005
Tau	Su et al., 1996a Dickson et al., 1999 Dickson and Vickers, 2001		
GAP43	Masliah et al., 1991 Masliah et al., 1992b	Dickson et al., 2000	Meller et al., 1993 Li et al., 1996 Christman et al., 1997 Hou et al., 1998 Schmitt et al., 1999 King et al., 2001

GAP43, growth associated protein 43

following axotomy of cortical neurons maintained in long-term culture (Dickson et al., 2000; King et al., 2001; Chuckowree and Vickers, 2003; Dickson et al., 2005). In both models of axonal injury, the appearance of ring-like neurofilamentous structures precedes the appearance of bulb-like structures (Dickson et al., 2000; Chuckowree and Vickers, 2003). The proportion of NF triplet protein and α-internexin immunoreactive ring-like structures is relatively constant prior to their disappearance (Dickson et al., 2005). Thus, the continued presence of α-internexin ring-like DNs in end-stage AD may indicate a distinct subset of neurons that are damaged relatively late in AD progression (Dickson et al., 2005), whereas the subset of neurons immunoreactive for both α-internexin and the NF triplet protein may be affected relatively earlier. This potential staging of disease progression may explain why the neurons that selectively express NF triplet proteins are particularly susceptible to NFT formation (Bussière et al., 2003), as they are also the neurons initially compelled to respond to the damaging effects of Aβ plaque formation (Vickers et al., 2000). Alternatively, the NF triplet protein-labelled neurons may possess a specific cytoskeletal profile that contributes towards susceptibility to NFT formation, whereas those neurons that labelled only with α-internexin may degenerate more rapidly without developing classical intraperikaryal neurofibrillary pathology.

The bulbous and swollen processes labelled for NF triplet proteins after axotomy *in vivo* are strikingly similar to those observed associated with Aβ plaques in AD (King et al., 2001). The presence of axonal clubs following trauma in humans and experimental models of axonal injury has been well documented since early last century (for review see Sahuquillo and Poca, 2002). As axoplasmic flow continues following axotomy, these axonal clubs consist of accumulated organelles (see Sahuquillo and Poca, 2002), such as vesicular and multi-lamellar bodies and mitochondria (King et al., 2001). Ultrastructural analysis of Aβ plaque-associated DNs in AD cases found that they contain clear vesicles, abundant mitochondria, multivesicular and lamellar bodies and filaments, which are characteristic of reactive, regenerative and dystrophic axonal changes in response to injury (Masliah et al., 1993). Meanwhile, other DNs show ultrastructural changes consistent with degenerating neurons (Masliah et al., 1993).

1.4 β-AMYLOID PLAQUES DISRUPT THE NEUROPIL

1.4.1 More compact/fibrous Aβ plaques are associated with neuritic pathology

The different morphological types of Aβ plaques in AD arise independently and affect the surrounding neurites to varying degrees (Dickson et al., 1988; Armstrong, 1998; Dickson and Vickers, 2001). β-amyloid plaques can be categorised as 'spherical diffuse', 'fibrillar' or 'dense-cored' depending on their three dimensional structure and packing density of Aβ fibrils (Dickson and Vickers, 2001). *In vivo* imaging provides evidence that Aβ plaques in Tg2576 mice (Table 1.2) are reasonably stable structures (Christie et al., 2001; Spires et al., 2005). Over extended periods of time, the vast majority of Aβ plaques do not change in size, however, new Aβ plaques do sometimes appear and rare Aβ plaques grow or shrink (Christie et al., 2001). The proportion of diffuse, fibrillar and dense-core Aβ plaques is different in preclinical AD and AD cases (Fukumoto et al., 1996; Dickson and Vickers, 2001). There is a lower proportion of diffuse and dense-core Aβ plaques in AD cases compared to preclinical AD cases, whereas the proportion of fibrillar Aβ plaques is increased in AD cases (Dickson and Vickers, 2001). In addition, the percentages of diffuse and fibrillar Aβ plaques associated with DNs are lower in preclinical stages as compared to the later stages of AD (13-24% and 47-82%, respectively), while the percentage of dense-core Aβ plaques that are neuritic is slightly decreased in AD compared to preclinical AD cases (82-76%; Dickson and Vickers, 2001). Overall, AD brains contain a higher relative proportion of fibrillar and dense-core Aβ plaques than preclinical AD cases, and it is these Aβ plaque types that are more regularly associated with DNs both in human brains and in transgenic mouse models of AD (Dickson et al., 1988; Fukumoto et al., 1996; Su et al., 1996a, Dickson and Vickers, 2001; Brendza et al., 2003; Noda-Saita et al., 2004; Dickson et al., 2005). Similarly, *in vitro* experiments show that fibrillar, but not amorphous Aβ, results in DN formation, somatic shrinkage and significant synaptic and neuron loss in hippocampal cultures (Pike et al., 1993; Lorenzo and Yanker, 1994; Ivins et al., 1998). These data are consistent with the hypothesis that it is the more densely packed, highly fibrillar Aβ plaques that are likely to cause the displacing and

Table 1.2 Transgene expression and genetic background of mouse models of AD.

Type	Name	Transgene/ promoter	Genetic Background	Primary reference
APP	**Tg2576**	human APP695 with Swedish (K670N/M671L) mutation/ hamster prion protein gene promoter	C57BL/6 APPsw x B6SJL F1	Hsiao et al , 1996
	PDAPP Line 109	human APP minigene with Indiana (V717F) mutation/ platelet derived growth factor-β	C57B6 x DBA2 F1 hybrid	Games et al , 1995
	APP23	human APP751 with Swedish (K670N/M671L) mutation/ Thy1 2 promoter	C57BL/6J	Sturchler-Pierrat et al , 1997
	TgCRND8	human APP with Swedish (K670N/M671L) and Indiana (V717F) mutations/ Syrian hamster prion promoter	Hybrid C3H/ He-C57BL/6	Chishti et al , 2001
	APPLon	human APP770 with Swedish (K670N/M671L) and London (V717I) mutation/ murine Thy1 promoter	FVB and C57B1/6	Moechars et al , 1999
	PDAPPSwe/ Ind (J20)	hAPP with Swedish (K670N/M671L) and Indiana (V717F) mutations/ platelet derived growth factor β promoter	C57BL/6 x DBA/2 F1	Mucke et al , 2000
	APPSw/Ind /Arc	human APP with Swedish (K670N/M671L), Indiana (V717F) and Arctic (E22G) mutations/ platelet derived growth factor β promoter	C57BL/6J	Cheng et al , 2004
Tau	**rTg (tauP301 L) 4510**	human 4R tau with (P301L) mutation/ calcium calmodulin kinase promoter system with a tetracycline-operon	129S6, FVB/N	SantaCruz et al , 2005
Double	**PSAPP**	Tg2576 x PS1 Line 5 1 (Duff et al , 1996), human APP695 with Swedish (K670N/M671L) mutation x human PS1 (M146L)/ hamster prion protein gene promoter and platelet derived growth factor β2, respectively	(C57BL/6 APPsw x B6SJL F1) x (Swiss Webster x B6D2F1)	Holcomb et al , 1998
	APPSwe/ Tauv/w	Tg2576 mice (Hsiao et al , 1996) x human 4R tau with mutations (G272V, P301L, R406W) / hamster prion promoter	(C57BL6xSJL) x (C57BL6xCBA)	Perez et al , 2005
	TAPP	Tg2576 mice (Hsiao et al , 1996) x human tau with mutation (P301L) JNPL3 (Lewis et al , 2000)/ hamster prion protein promoter, murine prion promoter, respectively	(C57BL/6 APPsw x B6SJL F1) x C57BL/DBA2/SW	Lewis et al , 2001
Triple	**3xTg-AD**	human APP695 with Swedish (K670N/M671L) mutation, human four repeat tau with the P301L mutation and PS1 M146V/ Thy-1 2 promoter	129/C57BL6	Oddo et al , 2003a

compressive forces sufficient to damage axons.

There is a decrease in the density of axons and dendrites within Aβ plaques compared to the surrounding neuropil (DeWitt and Silver, 1996; Knowles et al., 1998; Le et al., 2001; Tomidokoro et al., 2001; Adlard and Vickers, 2002; Moolman et al., 2004; Tsai et al., 2004). Specific types of Aβ plaques, defined by their morphology and packing density, affect normal axons and dendrites differentially (Le et al., 2001; Adlard and Vickers, 2002). Diffuse Aβ plaques do not affect the density of dendritic labelling in either AD or preclinical AD brains (Knowles et al., 1998; Adlard and Vickers, 2002). However, more fibrillar Aβ plaques exhibit significantly decreased dendrite labelling within the Aβ plaque area in comparison to the surrounding neuropil (Le et al., 2001; Adlard and Vickers, 2002). Interestingly, the density of dendrites within fibrillar Aβ plaques is not significantly decreased in preclinical AD cases (Adlard and Vickers, 2002). Specifically, the dendrites that remain within fibrillar and dense core Aβ plaques exhibit decreasing calibre, spine density, and deflect around the Aβ plaque and while other dendrites terminate (appear to be 'clipped') at the Aβ plaque margins (Adlard and Vickers, 2002; Grutzendler et al., 2007). Additionally, dendrites within Aβ plaques that are free of NFTs and neuropil threads have dramatically and significantly increased curvature (Knowles et al., 1999b). Furthermore, observations of the deflection of dendrites around dense and fibrillar Aβ plaques supports the concept that densely packed Aβ plaques act as space-forming lesions (Adlard and Vickers, 2002).

In vivo imaging of Aβ plaques in combination with neuronal processes in Tg2576, PDAPP and PSAPP transgenic AD mice models (Table 1.2) shows that diffuse Aβ plaques subtly change neurite geometry, while fibrillar Aβ plaques dramatically alter neurite trajectories and result in dendritic spine loss, shaft atrophy and axonal variscosities (Le et al., 2001; D'Amore et al., 2003; Stern et al., 2004; Tsai et al., 2004; Spires et al., 2005; Garcia-Alloza et al., 2006; Grutzendler et al., 2007). All dendrites that pass through fibrillar Aβ plaques in PSAPP mice exhibit spine loss and reduced shaft diameter, and a significantly higher percentage of dendrites terminate in the vicinity of fibrillar Aβ plaques than further away (Tsai et al., 2004). This reduction in

the number of dendrites passing through fibrillar Aβ plaques in PSAPP mice is age-dependent and led to neurite breakage and the permanent disruption of neuronal connections in PSAPP mice (Tsai et al., 2004). Interestingly, even though the average thioflavine s-stained plaque radius in PDAPP mice is 10μm there appears to be an approximately 40-50μm zone surrounding the plaques in PDAPP and PSAPP mice in which nearly half of the neuronal processes are disrupted (D'Amore et al., 2003; Grutzendler et al., 2007). The presence of Aβ plaques and the alterations in geometry of Aβ plaque-associated neurites results in an impairment of the magnitude and precision of the evoked synaptic response of neurons to transcallosal stimuli in Tg2576 mice in comparison to wild type age-matched controls (Stern et al., 2004). As the data also suggests that Aβ plaques do not affect the overall levels of synaptic innervation, these results indicate that Aβ plaques disrupt the synchrony of convergent inputs and reduce the ability of neurons to successfully integrate and propagate information (Stern et al., 2004). However, a recent study asserts that the density of synaptophysin-labelled boutons is significantly decreased within 200μm of fibrillar Aβ plaques in Tg2576 mice (Dong et al., 2007). In addition, other investigators report that dendritic spine density is significantly decreased in Tg2576 compared to age-matched wild type controls prior to the deposition of Aβ plaques, suggesting that expression of the APP transgene may also contribute to dendritic spine loss independently of Aβ plaque formation (Jacobsen et al., 2006).

1.4.2 β-amyloid plaques associated with familial AD cases

There are two additional Aβ plaque types observed in hereditary AD cases that are associated with mutations in PS1, namely "inflammatory" and "cotton wool" Aβ plaques. Inflammatory Aβ plaques have recently been described in PS1 AD cases, and have dense cresyl violet-, silver- and thioflavine s-stained cores that are not immunoreactive for Aβ, tau, ApoE and PS1, and are also associated with reactive microglia and astrocytes (Shepherd et al., 2005). "Cotton wool" Aβ plaques are numerous large rounded diffuse Aβ plaques in the frontal cortex of AD cases with PS1 mutations, and are relatively free of tau-labelled neuritic and glial components, despite

the presence of many NFTs and neuropil threads in nearby neuropil (Houlden et al, 2000; Mann et al., 2001; Steiner et al., 2001; Kwok et al., 2003). However, fine neurites labelled with hyperphosphorylated tau (Steiner et al., 2001; Shepherd et al., 2004) and globular DNs labelled for non-hyperphosphorylated tau are associated with cotton wool Aβ plaques (Takao et al., 2002; Shepherd et al., 2004). Cotton wool Aβ plaque pathology is potentially related to abnormally high production of Aβ1-42 (Houlden et al., 2000; Steiner et al., 2001), which is mainly due to the PS1 mutations, and in many cases amyloid fibrils within these Aβ plaques are rare (reviewed in Tabira et al., 2002). However, not all cases with hereditary AD caused by PS1 mutations exhibit cotton wool Aβ plaques (Janssen et al., 2001) and cotton wool Aβ plaques are also present in sporadic AD cases (Le et al., 2001).

1.4.3 Biochemical characteristics of neuritic and non-neuritic Aβ plaques

Recent research suggests that there may be biochemical differences in different Aβ plaque types, and between neuritic Aβ plaques and non-neuritic Aβ plaques. For instance, Aβx-40 immunoreactivity is mainly restricted to cored Aβ plaques, while diffuse Aβ plaques and cored Aβ plaques demonstrate immunoreactivity for Aβx-42 (Jimenez-Huete et al., 1998; Rábano et al., 2005). However, labelling with a novel antibody suggests that the principal component of diffuse Aβ plaques might be Aβx-17 peptides (Rábano et al., 2005). Interestingly, when transgenic mice that selectively express high levels of Aβ1-40 are crossed with mice selectively expressing Aβ1-42 or Tg2576 mice, the double transgenic mice show 60-90% decreases in Aβ plaque deposition, suggesting that Aβ1-40 inhibits Aβ deposition (Kim et al., 2007). In addition, an antibody that detects the internal residues Aβ11-16 specifically labels vascular amyloid and neuritic Aβ plaques in AD cases (Rábano et al., 2005). An antibody that specifically recognises the oligomeric Aβ structure has also relatively recently become commercially available, and should provide some interesting information regarding which species of Aβ is detrimental to neuronal health.

1.5 ABERRANT REGENERATION IN AD MAY BE RELATED TO THE STEREOTYPICAL RESPONSE OF NEURONS TO INJURY

Following axonal injury, a regenerative sprouting response is observed both *in vitro* and *in vivo* that is characterised by the presence of neurites expressing growth associated protein 43 (GAP43), APP and ApoE that share similarities with DNs in AD (Masliah et al., 1991; Otsuka et al., 1991; Masliah et al., 1992a, Masliah et al., 1992b; Benzing and Mufson, 1995; Li et al., 1996; Christman et al., 1997; Dickson et al., 1997; Thal et al., 1997; Hou et al., 1998; Dickson et al., 2000; Gómez-Ramos, et al., 2001; King et al., 2001; Chung et al., 2003; Sabo et al., 2003; Dickson et al., 2005) albeit, the DNs in AD have aberrant morphology. Accordingly, neurodegeneration in AD may be linked to abnormal stimulation of regenerative changes in neurons, leading to a re-expression of proteins and pathways linked to early neuronal and neurite development. Thus, neurodegeneration in AD may be closely linked to aberrant cellular plasticity.

1.5.1 Dystrophic neurites in AD are labelled for GAP43

In this regard, GAP43 has been widely used as a marker for neuritic outgrowth, including the regeneration of axons following injury (Masliah et al., 1992b; Li et al., 1996; Christman et al., 1997; Hou et al., 1998; Dickson et al., 2000; King et al., 2001). Sprouting neurites labelled with GAP43 and NF triplet protein are present following transection of axonal bundles *in vitro* (Dickson et al., 2000; King et al., 2001; Chuckowree and Vickers, 2003) and GAP43 messenger ribonucleic acid (mRNA) is upregualted in neurons in Clarke's nucleus following axotomy *in vivo*, even if they are destined to degenerate (Schmitt et al., 1999). GAP43-labelled Aβ plaque-associated DNs and GAP43 immunoreactive fine caliber neurites are also present in AD cases (Masliah et al., 1991; Masliah et al., 1992b), with approximately 50% of thioflavin s-stained plaques in AD cases containing GAP43-labelled neurites (Masliah et al., 1991). This sprouting response appears to be more prominent in the hippocampus of AD patients than in the neocortex as approximately 80% of thioflavine s-stained plaques in the hippocampus are associated with GAP43-labelled neurites (Masliah et al., 1991).

Additionally, fine fibrillar staining for GAP43 is increased in the neuropil in the AD hippocampus; especially in areas with decreased synaptophysin-labelling (Masliah et al., 1991) suggesting a sprouting response to Aβ plaques is associated with dendritic loss (Adlard and Vickers, 2002).

1.5.2 β-amyloid precursor protein is present in DNs in AD

The presence of APP in AD brains may also indicate the occurrence of post-injury regenerative processes. The APP is thought to play an important role in neurite outgrowth in development and following injury, with evidence suggesting that α-secretase cleaved APP and the APP intracellular domain may be neuroprotective and improve memory and long-term potentiation (Goodman and Mattson, 1994; Furukawa et al., 1996; Stein et al., 2004; Ma et al., 2007a; Ring et al., 2007). β-amyloid precursor protein immunoreactive spherical and club-like axonal swellings are widely used as a marker for axonal injury following various models of traumatic brain injury (Stone et al., 2001). For example, APP is present in neurons following a weight-drop model of traumatic brain injury (Lewen et al., 1995), in axons following an impact-acceleration head injury model of diffuse traumatic brain injury (Marmarou et al., 2005), and in axons and dystrophic axonal swellings after needle stab injuries in the rat brain (Otsuka et al., 1991). The co-localisation of GAP43, NF triplet protein and APP in a subpopulation of outgrowing neurites in the neonatal rat (Masliah et al., 1991; Masliah et al., 1992a) and the concentration of an APP complex that is likely to have a role in membrane motility regulation in the growth cones of neurons (Sabo et al., 2003), both suggest a role for APP in neuritic outgrowth. β-amyloid precursor protein immunoreactive Aβ plaque-associated DNs and pyramidal cell bodies are also present in AD (Masliah et al., 1992b). In AD over half of the DNs associated with Aβ plaques are intensely immunoreactive for APP and APP-labelling is also more intense in pyramidal neurons in the frontal cortex of AD patients than in control brains (Masliah et al., 1992b). Additionally, the increase in APP levels that occur in aged rat motor neurons following axonal injury (Sola et al., 1993; Xie et al., 2003) is associated with increased survival of these cells (Xie et al., 2003). As pyramidal cells appear to be selectively

vulnerable to degeneration in AD (Morrison et al., 1987; Hof, 1997; Vickers et al., 1997; Bussière et al., 2003), increased APP in pyramidal neurons in AD may indicate an increase in regenerative neuritic plasticity in this vulnerable cell population.

1.5.3 Apolipoprotein E is associated with several of the pathological hallmarks of AD

There is also evidence to suggest that ApoE has an important role in the development and regeneration of the nervous system (Poirier, 1994; Masliah et al., 1995; Strittmatter and Roses, 1995; Masliah et al., 1996a), however, the role of ApoE in AD is still unclear. ApoE plays important roles in the development, regeneration and aging processes of the nervous system (for review see Masliah et al., 1996a) probably due to its involvement in cholesterol uptake and redistribution and its putative role in the stabilisation of microtubules (Strittmatter and Roses, 1995). *In vitro* ApoE alleles apoε2 and ε3, but not ε4, bind to the microtubule binding domains of microtubule associated protein-2 (MAP2) and tau, and may stabilise the interactions of these two microtubule associated proteins with tubulin (Strittmatter and Roses, 1995). In support of this, the neurite outgrowth of a neuronal cell line was increased by the presence of apoε3 but not by apoε4 (Holtzman et al., 1995), and apoε3, but not apoε4, expression fully restored mossy fibre sprouting in ApoE knockout hippocampal slice cultures (Teter et al., 1999) and increased post-injury sprouting in the entorhinal cortex (White et al., 2001). Meanwhile, the delayed reinnervation of the dentate molecular layer after perforant pathway interruption in ApoE knock out mice strongly suggests a specific role for ApoE in regeneration following injury (Masliah et al., 1994). Furthermore, a neuroprotective role for ApoE has also been intimated, as the expression of human apoε3, but not apoε4, in $ApoE^{-/-}$ mice protects neurons from excitotoxic damage and age-dependent neurodegeneration (Buttini et al., 1999).

ApoE-labelled Aβ plaques, NFT-bearing neurons and possibly DNs are also present in AD (Benzing and Mufson, 1995; Dickson et al., 1997; Thal et al., 1997; Gómez-Ramos, et al., 2001). ApoE is present in all neuritic Aβ plaques in AD and preclinical AD cases

and some Aβ plaques that are not associated with DNs, suggesting that either ApoE enhances the ability of certain Aβ plaques to damage the surrounding neuropil or that ApoE is deposited in certain Aβ plaques after axonal injury during the process of attempted neurite regeneration (Dickson et al., 1997). As no fibrillar Aβ plaques or neuritic degeneration is observed in the brains of knockout ApoE mice expressing mutated human APP it is more likely that ApoE facilitates the formation of fibrillar Aβ plaques and the associated neuritic pathology (Holtzman et al., 2000). Indeed, intracerebral administration of lentiviral vectors expressing apoε4 and apoε2 in PDAPP mice increases and decreases hippocampal Aβ burdens, respectively (Dodart et al., 2005). ApoE-labelled DN-like structures that are not immunoreactive for tau, ubiquitin or NF triplet proteins are also observed in AD and preclinical AD cases (Dickson et al., 1997). While one study reports that nearly all ApoE-labelled cells in the nucleus basalis of Meynert and layer II of the entorhinal cortex of AD are co-localised with PHFs of tau (Benzing and Mufson, 1995), other studies found that ApoE is only occasionally co-localised with pre-NFT PHFs and intracellular NFTs (Dickson et al., 1997; Gómez-Ramos, et al., 2001). This suggests that ApoE mainly binds to NFTs once they are in the extracellular space, perhaps in a comparable way to the binding of ApoE to extracellular Aβ plaques (Gómez-Ramos et al., 2001). However, mice expressing apoε4 under the neuron specific enolase promoter develop intraneuronal inclusions of phosphorylated tau that are barely detectable in mice expressing apoε3 under the same promoter (Bretch et al., 2004).

1.5.4 Chronic aberrant plasticity may result in neurofibrillary pathology

A high phosphorylation state of tau is thought to be crucially involved in the formation of PHFs, which are the major constituents of NFTs and neuropil threads (Arendt, 2003a). Interestingly, a highly phosphorylated form of tau similar to PHF-tau is present distinctly in association with synaptic plasticity. This PHF-like tau is increased in animals during hibernation and arousal in association with the regression and re-establishment of synaptic contacts of mossy fibres with hippocampal cornu ammonis 3 (CA3) apical dendrites (Arendt et al., 2003). Foetal tau also exists in a similar

phosphorylation state to PHF-tau (Trojanowski and Lee, 1994). These findings point towards a high phosphorylation state of tau being a physiologically normal event that is associated with neuronal plasticity (Trojanowski and Lee, 1994; Arendt et al., 2003).

Perhaps the chronic stimulation of regenerative processes that require neuronal plasticity and place the neuron under metabolic stress in the AD brain result in the formation of an abnormally phosphorylated form of tau that leads to the formation of PHFs and consequently NFTs. Multiple kinases including activated microtubule-associated protein kinase, microtubule affinity regulating kinase, glycogen synthase kinase 3β (GSK3β), cyclin-dependent kinase 5 (cdk5) and cdc2/cyclin B1 kinase co-localise with PHF-tau suggesting that these kinases may be responsible for the abnormal phosphorylation of tau (Yamaguchi et al., 1996; Vincent et al., 1997; Knowles et al., 1999a; Chin et al., 2000; Noble et al., 2003). There is growing evidence that the dysregulation of cdk5 activation contributes to the abnormal phosphorylation of tau and perhaps the production of Aβ in AD, with an emerging role for cdk5 in APP processing (as reviewed in Lau and Ahlijanian, 2003; Cruz and Tsai, 2004). Ischemic or oxidative damage, Aβ treatment and other neurotoxic stimuli can elevate intracellular calcium and activate calpain (Cruz and Tsai, 2004), which can cleave cdk5 activator p35 into a p25 cleavage product that causes prolonged activation of cdk5 (Tseng et al., 2002) due to its longer half life (Cruz and Tsai, 2004). The ratio of p25 to p35 is higher in the AD hippocampus, frontal, inferior and parietal cortices than in control brains (Tseng et al., 2002). Furthermore transgenic mice overexpressing cdk5 activator p25 and mutant human tau accumulate significantly more aggregated tau and NFTs than tau-only transgenic mice, along with tau hyperphosphorylation at several sites (Noble et al., 2003). Additionally, data suggests that stress activated protein kinases, which may be activated in Aβ plaque-associated neurites in AD, are a likely candidate for the pathological phosphorylation of tau proteins (Buée et al., 2000).

1.5.5 Chronic neuronal plasticity may also result in loss of differentiation control and cell cycle re-activation in AD

It has also been proposed that the continuous need for neuronal plasticity and re-organisation in AD results in aberrant neuritic outgrowth (Arendt, 2003). For example, many GAP43-labelled ectopic entorhinal boutons associated with Aβ plaques are observed in the hippocampus, thalamus and white matter tracts of aged APP23 mice (Table 1.2; Phinney et al., 1999). It has further been postulated that this is due to de-differentiation of neurons in AD and the re-expression of developmental proteins in neurons that are involved in morphoregulation (Arendt, 2001; Arendt, 2003). There is accumulating evidence that proteins associated with AD such as APP, ApoE and the presenilins play an important role in the regulations of neuronal morphology (Arendt, 2003; Ji et al., 2003). Furthermore, loss of differentiation control, progressive morphodysregulation and cell cycle re-activation have all been linked to cell death in AD (Arendt, 2001). Cell cycle regulatory molecules including cdc2, cdk4, proliferating cell nuclear antigen, cyclin B and cyclin D, are re-expressed in neurons in AD but are rarely observed or not observed at all in control brains (McShea et al., 1997; Vincent et al., 1997; Busser et al., 1998; Ogawa et al., 2003; Yang et al., 2003). Specifically, cyclin B and cdc2 co-localise with each other and their M-phase specific tau-phosphoepitopes in hippocampal neurons with NFTs and neurons susceptible to NFT formation in AD cases (Vincent et al., 1997; Busser et al., 1998). Additionally, aged mice expressing non-mutant human tau in the absence of mouse tau develop NFTs and are labelled for cyclin D_1, ki67 and proliferating cell nuclear antigen, while the brains from age-matched wild type controls are negative for NFTs and these cell-cycle regulating proteins (Andorfer et al., 2005). Of interest is a recently developed transgenic mouse that conditionally expresses the simian virus 40 large T antigen oncogene, which forces cell cycle activation in post-mitotic neurons (Park et al., 2007). These mice exhibit tau phosphorylation, pre-tangles, diffuse Aβ plaque deposition, although a small number of fibrillar Aβ plaques, neurodegeneration and cell cycle changes similar to those in human AD supporting the suggestion that aberrant cell cycle activation may generate AD pathology (Park et al., 2007).

With respect to the underlying hypothesis that Aβ plaques cause structural injury to axons, the cell-wide response of neurons to damage involves sprouting and regenerative changes (Vickers et al., 2000). The persistence of the Aβ plaque structure and its interference with a 'normal' regenerative response may all contribute to 'pushing' neurons into an aberrant regenerative state, leading to profound cytoskeletal changes, characteristic tau modifications and, ultimately, neuronal degeneration.

1.6 ACCUMULATION OF AD-ASSOCIATED PATHOLOGY AND CELL DEATH IN AD

AD-associated hallmarks tend to appear first in the limbic and basal areas in the frontal, temporal and occipital cortices, and subsequently spread to the remaining areas of the cerebral cortex (Braak and Braak, 1991). However, the temporal and spatial pattern of NFT and neuropil thread distribution is different to that of Aβ plaque deposition (Braak and Braak, 1991; Lue et al., 1996; Knowles et al., 1998; Price et al., 1999). Neurofibrillary tangles and neuropil threads first appear in the entorhinal cortex and hippocampus, followed by an inferior to superior spread of pathology throughout the association cortices, and subsequently throughout the primary cortical areas (Pearson et al., 1985; Lewis et al., 1987; Arnold et al., 1991; Braak and Braak, 1991). Additionally, NFTs are mainly localised to neocortical layers III and V (Pearson et al., 1985; Lewis et al., 1987; Arnold et al., 1991; Arriagada et al., 1992; Hof et al., 1992). In comparison, Aβ plaque deposition is first observed in the basal portions of the frontal, temporal and occipital lobes, and as AD progresses deposition occurs in the hippocampus and all isocortical areas, with the primary cortices being affected last (Braak and Braak, 1991). β-amyloid plaques are deposited mostly in layer II, III, VI and the adjacent portion of layer V (Braak and Braak, 1991). Neuritic Aβ plaques also show a different pattern of distribution from that of NFTs, neuropil threads and Aβ plaques (as reviewed in Braak and Braak, 1997), and are predominantly localised to neocortical layers II, III, IV and V (Pearson et al., 1985; Lewis et al., 1987; Arnold et al., 1991). Interestingly, the patterns of neurofibrillary pathology and Aβ plaque distribution indicate that the populations of neurons that develop NFTs have neuronal processes that extend into the areas inhabited

by Aβ plaques (Hof, 1997). As the density of AD-associated pathology increases with disease progression, the limbic and basal isocortical areas in which pathological hallmarks first appear are the most severely affected in end-stage disease. However, there is still much debate as to whether it is the Aβ plaques or neurofibrillary pathology that initiates neuronal damage and degeneration in AD.

Overt neuronal loss occurs in the entorhinal cortex, hippocampus and neocortex of AD cases compared to control cases (Hof et al., 1990; Lippa et al., 1992; West et al., 1994; Fukutani et al., 1995; Gómez-Isla et al., 1996; Kril et al., 2002; Kril et al., 2004). In the entorhinal cortex, an area severely affected by AD pathology, a decrease of 45-75% in neuronal numbers occurs in AD cases compared to control cases, which show no age-related neuron loss (Lippa et al., 1992; Fukutani et al., 1995; Gómez-Isla et al., 1996). Similarly, a different pattern of neuron loss is detectable in the hippocampal subfields of AD cases compared to aged control cases, with the CA1 zone of the hippocampus being most severely affected (West et al., 1994; Kril et al., 2002; Kril et al., 2004). Some studies have stated that the degree of neuron loss in AD is equivalent to the extent of NFT formation (Hof et al., 1990; Cras et al., 1995; Fukutani et al., 1995; Cullen and Halliday, 1998), suggesting that NFT-formation may cause cell death. For instance, the overall loss of neurons in the entorhinal cortex is reported to correlate with the density of NFTs and neuritic Aβ plaques in some studies, while the dramatic loss of neurons in layer II and IV of the entorhinal cortex paralleled the specific accumulation of NFTs in these entorhinal laminae (Lippa et al., 1992; Gómez-Isla et al., 1996). In contrast, others assert that NFTs only account for 38-55% of variability in neuronal numbers in hippocampal areas in AD (Von Gunten et al., 2006). Additionally, when the numbers of intracellular and extracellular NFTs are assessed in the hippocampal CA1 and the superior frontal gyrus (SFG) and inferior temporal gyrus (ITG) of the neocortex, NFT formation seems likely to account for only a small proportion of neuron loss (Gómez-Isla et al., 1997; Kril et al., 2002; Vickers et al., 2003). In addition, Salehi and colleagues (1998) report that while neuronal density in areas surrounding neuritic Aβ plaques in the CA1 is 16-19% lower than in the unaffected neuropil, this would only

account for 2.6% of the neuronal loss that occurs in the CA1 region. Thus, Aβ plaques and NFTs may both contribute the neuronal cell death that occurs in AD.

1.6.1 Neurons that express NF triplet proteins are selectively vulnerable to degeneration in AD

The neuronal loss observed in AD affects certain sub-populations of neurons. Pyramidal neurons, especially those labelled with NF triplet proteins, are selectively vulnerable to degeneration in AD (Morrison et al., 1987; Hof et al., 1990; Vickers et al., 1992; 1994a; Hof, 1997; Vickers et al., 1997; Bussière et al., 2003). In comparison non-pyramidal cortical neurons, typically interneurons, expressing calcium-binding proteins such as parvalbumin, calretinin and calbindin are relatively resistant to AD pathology (Hof et al., 1990; Ferrer et al., 1991; Hof et al., 1991a; Hof et al., 1991b; Hof et al., 1993; Sampson et al., 1997; Leuba et al., 1998). Originally it was assumed that calcium binding proteins were neuroprotective, but the discovery of a subset of calretinin-labelled neurons that are also labelled for NF triplet proteins and are susceptible to NFT formation, lead to the suggestion that NF triplet protein alterations may enable or be necessary for NFT formation and/or degeneration (Vickers et al., 1992; Vickers et al., 1994; Vickers et al., 1996; Sampson et al., 1997).

1.7 MECHANISM OF CELL DEATH IN AD

Despite evidence of neuron loss in the AD brain, there are numerous, varied and opposing views regarding exactly how these neurons die. The most prominent premise is that neuronal death occurs via apoptosis. The next section of this review details the characteristics of apoptosis and the *in vitro* and *in vivo* data regarding neuronal apoptosis in AD. In addition, the data regarding neuronal apoptosis in AD is not at odds with the "mass effect" hypothesis, as apoptotic changes have also been observed in neurons following neurite injury (Berkelaar et al., 1994; Pravdenkova et al., 1996; Hou et al.,

1998; Springer et al., 1999; Büki et al., 2000; Smith et al., 2000; Wingrave et al., 2003; DeRidder et al., 2006).

1.7.1 The morphological and biochemical characteristics of apoptosis

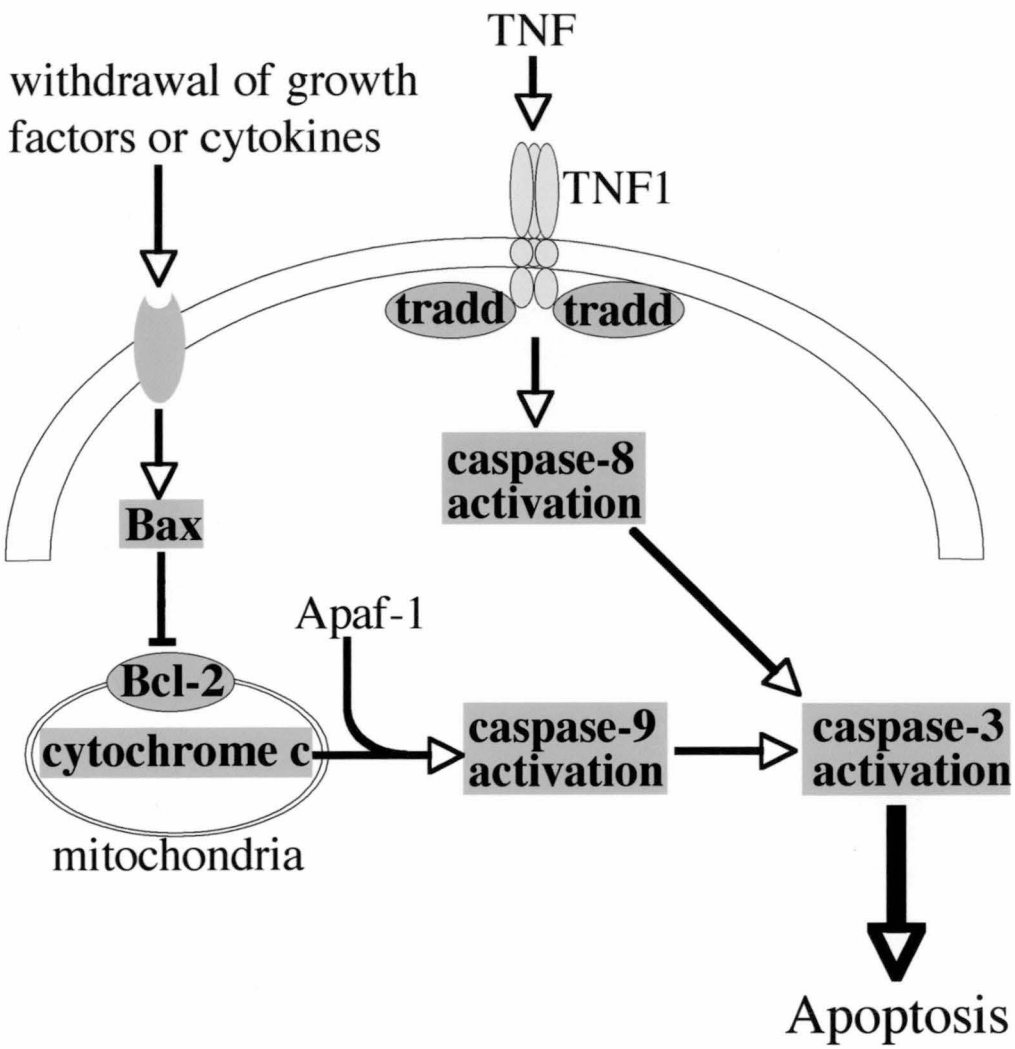
Programmed cell death has been described and defined by many overlapping schemas; as apoptotic, autophagic, necrotic, energy-dependent and energy-independent, active and passive. While apoptotic, autophagic and necrotic cell death were once thought of as being separate and defined cell death programs, there is now increasing evidence that these cell death programs are not mutually exclusive, but may occur as a continuum and even co-exist in the same cell (as reviewed in Bursch, 2004; Stefanis, 2005). Morphologically and biochemically apoptosis involves cytoplasmic shrinkage, chromatin condensation, deoxyribose nucleic acid (DNA) fragmentation, early dissolution of the nucleolus, alterations of cell membrane composition, membrane blebbing, the formation of apoptotic bodies (Su et al., 1996b; Bratton et al., 2000) and caspase-activation (Stefanis, 2005). Caspases are a family of cysteine proteases that are activated in a cascade-like fashion and cleave key cellular proteins that result in apoptotic cell death. Two pathways activate the apical caspases of this cascade: the intrinsic or mitochondrial pathway and the extrinsic or death receptor pathway (Figure 1.1).

In the intrinsic apoptotic pathway cytochrome c (cyto c) is released from the mitochondria and associates with Apaf-1, dATP and caspase-9 to form the cytoplasmic apoptosome complex. Formation of the apoptosome complex then results in the activation of caspase-9 and the subsequent activation of effector caspases such as caspase-3. Release of cyto c from the mitochondria is regulated by the Bcl-2 family of proteins, which includes both pro-apoptotic (Bax, Bad, Bak, Bid) and anti-apoptotic (Bcl-2, Bcl-x_L) members. In turn, the Bcl-2 family is regulated by cytokines and other death-survival signals such as the p53-mediated damage response (as reviewed in Adams and Cory, 2001). Anti-apoptotic Bcl-2 and Bcl-x_L inhibit the release of cyto c by blocking the opening of the mitochondrial permeability transition pore, whereas pro-

Figure 1.1

Apoptosis can be initiated through the activation of either the intrinsic or extrinsic apoptotic pathways. The intrinsic or mitochondrial apoptotic pathway is regulated by the relative levels of pro- (eg. Bax) and anti-apoptotic (eg. Blc-2) proteins, which are members of the Bcl-2 protein family. In turn, the Bcl-2 family of proteins are regulated by cytokines and other death-survival signals. Pro-apoptotic Blc-2 family members inhibit the opening of the mitochondrial permeability transition pore, while pro-apoptotic Bcl-2 family members induce the opening of mitochondrial membrane pores. If pores in the mitochondrial membrane are opened cyto c is released from the mitochondria and associates with Apaf-1, dATP and caspase-9, which results in the activation of caspase-9, and the subsequent activation of effector caspases such as caspase-3.

The death receptor or extrinsic apoptotic pathway involves extracellular ligands binding to death receptors in the cell membrane (eg. Fas, Tumour necrosis factor receptor 1, TNFR1). Ligands binding to death receptors cause conformational changes in the receptors, resulting in the recruitment of various adaptor proteins to form several death receptor complexes. Different death receptor complexes recruit and activate different apical caspases, which then activate effector caspases such as caspase-3. For instance, the activated TNFR1 recruits TNF receptor-associated death domain (TRADD), which can recruit Fas associated death domain (FADD) and caspase-8, resulting in the activation of caspase-8.


TNF
withdrawal of growth
factors or cytokines
TNF1
tradd
tradd
caspase-8
activation
Bax
Apaf-1
Bcl-2
cytochrome c
caspase-9
activation
caspase-3
activation
mitochondria
Apoptosis

apoptotic Bcl-2 family members induce the opening of pores in the mitochondrial membrane including the permeability transition pore (as reviewed in Jordan et al., 2003; Tatton et al., 2003). Bcl-x_L may also bind to Apaf-1 and prevent it from activating caspase-9 (Adams and Cory, 2001). Thus, the balance of pro-apoptotic and anti-apoptotic Bcl-2 family members may be critical to the survival of an individual cell (Shimohama, 2000).

Activation of the death receptor or extrinsic pathway involves the binding of extracellular ligands to death receptors in the cell membrane, which cause conformational changes in the receptors and the formation of intracellular death protein complexes. These death protein complexes then recruit and activate caspase-8, which subsequently activates effector caspases. Death receptors are members of the tumour necrosis factor receptor (TNFR) superfamily and include Fas, TNFR1/2 and TNF-related apoptosis-inducing ligand-receptors 1/2 (Bratton et al., 2000). Different receptors recruit different adaptor proteins and form different death receptor complexes that can recruit and activate different caspases. For instance, the ligand bound Fas receptor can recruit Fas associated death domain (FADD) proteins that associate with caspase-8 (Muzio et al., 1998). Whereas the activated TNFR1 can recruit TNF receptor-associated death domain (TRADD), which recruits FADD and caspase-8, or TRADD can bind receptor-interacting protein, which recruits receptor-interacting protein-associated ICH-1/CED-3 homologous protein with a death domain/caspase receptor-interacting protein with a death domain and then caspase-2 (Duan et al., 1997). There is also some cross talk between the intrinsic and extrinsic apoptotic pathways in some cell types, as activated caspase-8 can cleave and activate Bcl-2 family member Bid, which translocates to the mitochondria and induces cyto c release (Stefanis, 2005).

1.7.2 Apoptosis in neurons

Neurons predominantly use the intrinsic pathway of apoptosis, but in some cases neurons can use the extrinsic pathway or undergo autophagy, caspase-independent non-apoptotic cell death involving other proteases (eg. calpains), mitochondrial alterations

(eg. energy depletion) or the generation of free radicals and permeability transition pore opening that releases cytotoxic proteins (as reviewed in Stefanis, 2005). Apoptotic pathways may also differ depending on the death stimulus and cell type, so that the presence of caspase-activation or the morphological features of apoptosis do not always equate to cell death (Stefanis, 2005). Interestingly, there is also increasing evidence that caspases may have apoptosis-independent roles in proliferation, cell cycle regulation, differentiation and other cellular processes, perhaps by cleaving a limited number of selected substrates (as reviewed by Schwerk and Schulze-Osthoff, 2003).

1.7.3 β-amyloid-induced neuronal apoptosis *in vitro*

Many *in vitro* models have shown that treating primary neuronal cultures and cell lines with fibrillar or soluble Aβ or fragments of Aβ result in apoptotic cell death (Yanker et al., 1990; Forloni et al., 1993; Loo et al., 1993; Pike et al., 1993; Lambert et al., 1994; Gschwind and Huber, 1995; Pike et al., 1995; Paradis et al., 1996; Estus et al., 1997; Lambert et al., 1998; Ivins et al., 1999; Pillot et al., 1999; Selznick et al., 2000; Morishima et al., 2001; Jang and Surh, 2002; Wei et al., 2002; Cantarella et al., 2003; Hoshi et al., 2003; Lu et al., 2003; Sola et al., 2003; Sponne et al., 2003; Gestwicki et al., 2004; Movsesyan et al., 2004; Paradisi et al., 2004; Bloom et al., 2005; Caraci et al., 2005; Jang and Surh, 2005; Millet et al., 2005; Ohyagi et al., 2005; Ramírez et al., 2005; Yao et al., 2005; St John, 2007). Some studies indicate that Aβ induces apoptosis via the intrinsic pathway (Paradis et al., 1996; Selznick et al., 2000; Sola et al., 2003; Keil et al., 2004; Movsesyan et al., 2004; Caraci et al., 2005; Jang and Suhr, 2005; Ohyagi et al., 2005; Yao et al., 2005; Biswas et al., 2007), while other studies present evidence that it acts via the extrinsic apoptotic pathway (Ivins et al., 1999; Wei et al., 2002; Cantarella et al., 2003; Lu et al., 2003; Su et al., 2003; Suen et al., 2003). There is also disagreement as to whether it is soluble oligomeric/protofibrillar (Roher et al., 1996; Lambert et al., 1998; Hartley et al., 1999; Pillot et al., 1999; Hoshi et al., 2003; Blanchard et al., 2004; Barghorn et al., 2005; Piccini et al., 2005; Walsh et al., 2005; Whalen et al., 2005), or aggregated/fibrillar (Forloni et al., 1993; Loo et al., 1993; Pike et al., 1993; Lorenzo and Yanker, 1994; Howlett et al., 1995; Pike et al., 1995; Paradis et

al., 1996; Estus et al., 1997; Walsh et al., 1999; Gestwicki et al., 2004; Paradisi et al., 2004; St John, 2007) Aβ that is the neurotoxic species of Aβ.

Although data suggests that Aβ causes neuronal apoptosis *in vitro* this may not be representative of the *in vivo* milieu. *In vitro* models are often not representative of the *in vivo* environment due to the use of supra-physiological concentrations of Aβ, the lack of glia-neuron interactions and the use of relatively immature neurons. The concentration of synthetic Aβ or Aβ fragments used to treat neurons *in vitro* is typically around 20-30μM (Pike et al., 1992; Lorenzo and Yanker, 1994; Estus et al., 1997; Pillot et al., 1999; Paradisi et al., 2004; Caraci et al., 2005; Whalen et al., 2005; Yao et al., 2005), however, the level of Aβ present in the CSF of humans is 4nM (Paradis et al., 1996; Marques et al., 2003). Additionally, treating primary human neuronal cultures with 100nM fibrillar Aβ1-40 or Aβ1-42 results in little neuronal apoptosis (Paradis et al., 1996). Although, nanomolar concentrations of soluble Aβ oligomers can kill neurons (Lambert et al., 1998). Furthermore, most Aβ toxicity models do not account for the complex interactions between neurons and glia that occur *in vivo*. The rate of neuronal death differs following Aβ treatment when neurons are cultured alone compared to when they are co-cultured with astrocytes or microglia (Roher et al., 1996; Paradisi et al., 2004; Caraci et al., 2005; Ramírez et al., 2005). Many *in vitro* models also utilise primary neurons that are cultured for up to one day (Pike et al., 1992; Pike et al., 1993; Pike et al., 1995; Gestwicki et al., 2004), two to six days (Yanker et al., 1990; Loo et al., 1993; Lorenzo and Yanker, 1994; Ivins et al., 1999; Sponne et al., 2003; Paradisi et al., 2004; Whalen et al., 2005; Yao et al., 2005) or seven to 10 days (Paradis et al., 1996; Pillot et al., 1999; Sponne et al., 2003; Ramírez et al., 2005; Whalen et al., 2005) *in vitro* before the Aβ treatment is initiated. Recent results indicate that primary neuron cultures develop a more mature phenotype when they have spent 21 days *in vitro* (King et al., 2006). Treating primary neuron cultures with concentrations of Aβ that are more similar to those *in vivo* and aging neuron cultures for at least 21 days *in vitro* may provide a more accurate model in which to assess the toxic effects of Aβ on neurons.

1.7.4 Apoptosis in AD

The final pathway to cell death in AD may involve NFT formation, cell cycle or plasticity dysregulation, apoptosis or cell death that is neither classical necrosis nor apoptosis (reviewed in Vickers et al., 2000; Jellinger and Stadelmann, 2001). To ascertain whether apoptosis is involved in AD-associated degeneration studies have focused on the presence of DNA fragmentation, frank apoptotic morphology, changed levels of pro- and anti-apoptotic proteins, caspases and caspase-cleavage products.

1.7.4.1 DNA fragmentation and apoptotic nuclear morphology in AD

Many studies report increased DNA fragmentation in the hippocampal formation, frontal, temporal, parahippocampal and entorhinal cortices of AD cases compared to controls (Su et al., 1994a; Dragunow et al., 1995; Smale et al., 1995; Cotman and Su, 1996; Lassmann, 1996; Troncoso et al., 1996; Lucassen et al., 1997; Sugaya et al., 1997; Masliah et al., 1998; Sheng et al., 1998; Stadelmann et al., 1998; Overmyer et al., 2000; Pompl et al., 2003). However, other studies observe similar levels of DNA fragmentation in the AD and control temporal cortex and variable levels in the occipital cortex (Lucassen et al., 1997). Meanwhile, classical apoptotic morphology is observed rarely or not at all in AD (Lassmann et al., 1995; Troncoso et al., 1996: Lucassen et al., 1997; Tompkins et al., 1997; Stadelmann et al., 1998; Stadelmann et al., 1999; Nunomura and Chiba, 2000; Jellinger and Stadelmann, 2000; Jellinger and Stadelmann, 2001; Raina et al., 2001; Raina et al., 2003). However, both DNA fragmentation and classical apoptotic morphology can occur independently of apoptosis (Kaasik et al., 1999; Raina et al., 2003; Tatton et al., 2003).

1.7.4.2 Apoptotic-related proteins in AD

Similar contrasting and conflicting results are also reported when the levels/and or the number of cells containing Bcl-2, Bax, active and inactive caspase-3, -8 and –9 are analysed in AD and control brains (Table 1.3). Interestingly, one study found no Bax in the membranous fraction of the temporal cortex of AD brains (Kitamura et al., 1998),

Table 1.3 Summary of the levels of apoptotic-related proteins present in AD brains compared to control brains.

Apoptotsis marker	Change AD vs control	Cerebral area	Method	Reference
caspase-9	Increased	entorhinal cortex	mRNA probe	Pompl et al , 2003
	Decreased	cerebellum	WB, immunolabelling	Egidawork et al , 2001
	No difference	frontal cortex	WB, immunolabelling	Egidawork et al , 2001
		nucleus basalis of Meynert	immunolabelling	Wu et al , 2005
active caspase-9	Increased	frontal cortex	WB	Lu et al , 2000
		hippocampus	immunolabelling	Rohn et al , 2002
caspase-8	Increased	entorhinal cortex	mRNA probe	Pompl et al , 2003
	Decreased	frontal cortex, cerebellum	Immunolabelling	Egidawork et al., 2001
	No difference	frontal cortex, cerebellum	WB	Egidawork et al., 2001
		nucleus basalis of Meynert	Immunolabelling	Wu et al., 2005
active caspase-8	Increased	hippocampus	Immunolabelling	Rohn et al , 2001a
caspase-3	Increased	neurons in frontal cortex	Immunolabelling	Masliah et al , 1998
		hippocampal neruons	Immunolabelling	Jellinger & Stadelmann, 2001
		entorhinal cortex	mRNA probe	Pompl et al , 2003
	Decreased	frontal cortex, cerebellum	WB, immunolabelling	Egidawork et al , 2001
	No difference	medial temporal lobe	Immunolabelling	Raina et al , 2001
		nucleus basalis of Meynert	Immunolabelling	Wu et al , 2005
active caspase-3	Increased	hippocampus, subiculum, temporal isocortex	immunolabelling	Stadelmann et al , 1999
		hippocampus, entorhinal cortex, temporal cortex	WB	Zhao et al , 2003a
		hippocampus, entorhinal cortex, SFG	WB, immunolabelling	Zhao et al , 2003b
		increased in cells of the cortex	Immunolabelling	Kang et al , 2005
	No difference	entorhinal cortex, hippocampus, subiculum	immunolabelling, mass spectroscopy	Gastard et al , 2003
Bax	Increased	hippocampus, subiculum, dentate gyrus	immunolabelling	Nagy and Esiri, 1997
		hippocampal formation	immunolabelling	Su et al., 1997
		NFT-free neurons in the hippocampal formation,	immunolabelling	Giannakopolous et al., 1999
		SFG and ITG	immunolabelling	Blanchard et al., 2003
	No difference	Hippocampal dentate granule cells	immunolabelling	MacGibbon et al., 1997
		frontal cortex	semi-quant RT-PCR	Desjardins and Ledoux, 1998
		cystolic fraction of temporal cortex	WB	Kitamura et al., 1998
		nucleus basalis of Meynert	immunolabelling	Wu et al., 2005
Bcl-2	Increased	entorhinal cortex, subiculum, hippocampus	immunolabelling	Satou et al., 1995
		hippocampal formation	immunolabelling	Su et al., 1996b
		membranous fraction, temporal cortex	WB	Kitamura et al , 1998
		hippocampal formation	immunolabelling	Su et al , 1997
		neurons in frontal cortex	immunolabelling	Masliah et al., 1998
	No difference	hippocampus, subiculum dentate gyrus	immunolabelling	Nagy and Esiri, 1997

Apoptotic Marker	Change AD vs Control	Cerebral area	Method	Reference
Bcl-2	No difference	frontal cortex	RT-PCR	Desjardins and Ledoux, 1998
		CA1, sublicular/entorhinal regions	immunolabelling	Stadelmann et al., 1998
TRADD	Increased	hippocampus, entorhinal cortex, SFG	WB, immunolabelling	Zhao et al., 2003b
		hippocampus	mRNA levels, immunolabelling	Del Villar and Miller, 2004

WB, western blotting

suggesting that Bax had not been translocated to the mitochondrial membrane. Increases in other less frequently studied apoptosis-associated proteins such as Bcl-x_L (Nagy and Esiri, 1997; Kitamura et al., 1998), Blc-x_β, (Kitamura et al., 1998), Bak (Kitamura et al., 1998), Bad (Kitamura et al., 1998), p53 (De la Monte et al., 1997), Fas/FasL (De la Monte et al., 1997; Su et al., 2003), TRADD (Zhao et al., 2003b; Del Villar and Miller, 2004), cyto c (Blanchard et al., 2003), X-inhibitors of apoptosis protein (IAP) (Christie et al., 2007), caspase-cleaved APP (Zhao et al., 2003a), caspase-cleaved fodrin (Rohn et al., 2001b), active caspase-6 (Guo et al., 2004) and caspase-1, -2L, -5, -6 and –7 mRNA (Pompl et al., 2003) are reported in AD or APP-overexpressing mice compared to control brains. However, other studies report that Bcl-x_L (Giannakopoulos et al., 1999), Bcl-x (Desjardins and Ledoux, 1998; Wu et al., 2005), Apaf-1 (Engidawork et al., 2001), cyto c (Engidawork et al., 2001), caspase-1 (Masliah et al., 1998), -6 and –7 (Raina et al., 2001), NIAP (Christie et al., 2007), cIAP-2 (Christie et al., 2007), and Fas/FasL (Ferrer et el., 2001; Wu et al., 2005) levels are unchanged or decreased. Additionally, when preclinical or early AD cases are compared to control cases increases in DNA fragmentation (Troncoso et al., 1996), levels of Bax (Nagy and Esiri, 1997: Su et al., 1997), Bcl-2 (Satou et al., 1995; Su et al., 1997), active-caspase-3 (aC3; Gastard et al., 2003; Zhao et al., 2003a) and caspase-1 and –7 mRNA (Pompl et al., 2003) and similar levels of TRADD (Zhao et al., 2003b) and NAIP (Christie et al., 2007) are detected.

It is also important to note that the presence of apoptotic markers in AD brains may not necessarily equate to apoptosis. For instance, if the presence of pro-apoptotic proteins or DNA fragmentation resulted in apoptosis, a substantial neuronal loss would occur within a short period of time (Perry et al., 1998). Thus, it is plausible that apoptotic pathways are activated in the cells exhibiting apoptotic changes but do not proceed to completion (Raina et al., 2001; Raina et al., 2003; Wu et al., 2005) due to sublethal activation of apoptotic pathways (as reviewed in Cotman, 1998) including a lack of apoptotic signal propagation to downstream effector caspases (Raina et al., 2001; Raina et al., 2003), or cells mounting an effective defence against apoptotic cell death (Perry et al., 1998; Raina et al., 2003). For instance, long-term survival of neurons labelled for activated

caspases is not unheard of. In a model of long-term experimental diabetes, sensory neurons express aC3, but show no signs of ongoing apoptosis in terms of DNA fragmentation, nuclear morphology or cell loss (Cheng and Zochodne, 2003). Finally, there is also evidence that aberrantly activated caspases can be present in neurons uncommitted to apoptosis, which over long periods, may lead to aberrant processing of proteins (LeBlanc et al., 1999) such as tau (Gamblin et al., 2003; Rissman et al., 2004; Kang et al., 2005) and APP (as reviewed in Tanzi, 1999).

1.7.4.3 Association between apoptosis-related changes and AD pathology

Investigations into the association of apoptotic-related changes with the pathological hallmarks of AD also yield variable results. Numerous studies have investigated the co-localisation of NFTs with apoptotic markers. A subset of NFT-bearing neurons also exhibit DNA fragmentation (Su et al., 1994a, Lassmann et al., 1995; Sugaya et al., 1997; Sheng et al., 1998; Broe et al., 2001; Jellinger and Stadelmann, 2001), apoptotic nuclear morphology (Su et al., 1994a), Fas/FasL (Ferrer et al., 2001), Bax (Giannakopolous et al., 1999; Blanchard et al., 2003), Bcl-2 (Tortosa et al., 1998), Bcl-x_L (Giannakopolous et al., 1999), FADD (Wu et al., 2005), caspase-cleaved tau (Guo et al., 2004), caspase-8 and –9 (Raina et al., 2001) and active caspase-3 (aC3, Gastard et al., 2003; Kang et al., 2005), -6 (Guo et al., 2004; Albrecht et al., 2007) and -8 (Rohn et al., 2001a; Su et al., 2002). However, DNA fragmentation (Su et al., 1994a; Lassmann et al., 1995; Sheng et al., 1998), apoptotic nuclear morphology (Su et al., 1994a), FADD (Wu et al., 2005), Fas/FasL (Ferrer et al., 2001), Bcl-2 (Tortosa et al., 1998) and aC3 (Gastard et al., 2003) are also present in a subset of the NFT-free neurons in AD. Caspase-8, -9 (Raina et al., 2001) and Bax-labelling (MacGibbon et al., 1997) is reportedly strong in NFT-bearing and weak in NFT free neurons. In contrast other studies assert that NFT-bearing neurons exhibit reduced or no labelling for Bax (Nagy and Esiri, 1997; Su et al., 1997), Bcl-2 (Satou et al., 1995; Su et al., 1996b), p53 (De la Monte et al., 1997) and TRADD (Del Villar and Miller, 2004), while pre-NFTs demonstrate strong labelling for Bax (Su et al., 1997) and weak labelling of Bcl-2 (Su et al., 1996b) and p53 (De la Monte et al., 1997). Yet other studies observe no difference between NFT-bearing and NFT-free

neurons for Bax and Bcl-2-labelling (Tortosa et al., 1998; Kobayashi et al., 2004). Additionally, co-localisation of NFTs and caspase-cleaved fodrin, APP and tau are also observed (Rohn et al., 2001b; Rohn et al., 2002; Zhao et al., 2003a; Rissman et al., 2004; Kang et al., 2005). Interestingly, it has been hypothesised that caspase activation may link Aβ and neurofibrillary pathology in AD, with Aβ activating caspases, which then cleave tau and initiate or potentiate NFT formation (see Jellinger, 2006).

The distribution of Aβ plaques is not correlated with DNA fragmentation and apoptotic-like nuclei (Lassmann et al., 1995; Lucassen et al., 1997; Broe et al., 2001), but DNA fragmentation is positively correlated with Aβ plaque load (Jellinger and Stadelmann, 2001). Bax-labelling is highly variable adjacent to Aβ plaques (Nagy and Esiri, 1997), although some studies describe Bax-labelled glia in association with Aβ plaques (MacGibbon et al., 1997; Su et al., 1997; Giannakopolous et al., 2001). Additionally, the number of Bcl-2-labelled neurons or intensity of Bcl-2-labelling present in the AD brain correlates with Aβ plaque load (Satou et al., 1995; Kobayashi et al., 2004). Bax, Bcl-x_L, cyto c, p53, FasL, FADD, caspase cleaved tau and active caspases-8 and –9 are also observed in a subset of DNs in AD (De la Monte et al., 1997; MacGibbon et al., 1997; Nagy and Esiri et al., 1997; Tortosa et al., 1998; Giannakopolous et al., 2001; Rohn et al., 2002; Su et al., 2002; Blanchard et al., 2003; Su et al., 2003; Guo et al., 2004; Wu et al., 2005; Albrecht et al., 2007).

The variability, discrepancies and contradictions present in the current literature regarding apoptosis in AD may be due to the different cerebral areas and cell populations analysed, the different techniques and/or antibodies used and even differences between fixation methods or the cohort of brain cases analysed (eg. Table 1.3). Although evidence supporting a direct role for apoptosis in AD degeneration has accumulated it remains difficult to draw conclusions from the current literature (Roth, 2001; Raina et al., 2003). A comprehensive analysis of several apoptotic-related proteins and apoptotic morphology in the same cohort of cases and their association with the pathological hallmarks of AD may shed light on this contentious area of AD research.

1.8 ANIMAL MODELS OF AD

Animal models present the opportunity to examine the effects of overexpression of disease associated genes and genetic mutations, disease progression and provide models in which potential therapeutics can be screened and tested. Now that initial difficulties in producing AD mouse models that closely resemble AD-pathological features (reviewed in Vickers et al., 2001) have been overcome, numerous mouse models of AD have been developed and many are now commercially available. Transgenic mouse models of AD express a variety of combinations of human APP alone or in combination with PS1/PS2 and/or tau proteins harbouring mutations associated with familial AD, and demonstrate diffuse and dense Aβ plaques, DNs, gliosis and neurodegeneration that are not observed in wild type littermates, but not neurofibrillary pathology or extensive neuronal death (Games et al., 1995; Hsiao et al., 1996; Borchelt et al., 1997; Sturchler-Pierrat et al., 1997; Holcomb et al., 1998; Moechars et al., 1999; Janus et al., 2000; Mucke et al., 2000; Chishti et al., 2001; Blanchard et al., 2003; Higgins and Jacobsen, 2003; Richards et al., 2003; Cheng et al., 2004; Kawasumi et al., 2004; Oakley et al., 2006). Transgenic tau mice have also been developed that express human tau harbouring mutations associated with hereditary parkinsonism and frontotemporal dementia (Lewis et al., 2000; Gotz et al., 2001; Zhang et al., 2004a; SantaCruz et al., 2005; Terwel et al., 2005). The most widely used mouse models of AD have differing characteristics and, thus, there are advantages and disadvantages associated with each mouse model.

1.8.1 β-amyloid precursor protein transgenic mouse models

There are many widely utilised mouse models of AD that express human APP with various familial associated mutations under the control of differing promoters (Table 1.2). The most frequently used APP-expressing AD mouse models include Tg2576 (APPSwe), PDAPP, APP23 and TgCRND8 mice. The expression of various familial associated APP mutations and the different levels and localisation of transgene

expression in AD mouse models result in variability in the temporal and region specific pattern of AD-associated pathology. However, differences between transgenic AD mouse models can be attributed not only to the different promoters and transgenes with varying disease-associated mutations, but the genetic background of the mouse in which the transgene is expressed also affects the phenotype (Ali et al., 1996; Carlson et al., 1997).

1.8.1.1 Tg2576 transgenic AD mice

Tg2576 mice were one of the first successful transgenic AD mouse models (Hsiao et al., 1996; Higgins and Jacobsen, 2003). Tg2576 mice develop age-dependent Aβ plaque deposition beginning at seven months of age, which progressively increases and is severe by 23 months of age (Kawarabayashi et al., 2001; Noda-Saita et al., 2004). Compared to other APP transgenic mice, Tg2576 mice accumulate pathology over a relatively long period of time, providing a mouse model that more closely resembles the chronic time course of human AD. β-amyloid plaques are present in the hippocampal formation and entorhinal cortex, cerebral cortex, cerebellum and cerebral vasculature of Tg2576 mice, with rare Aβ plaque deposition in the internal capsule and basal ganglia (Hsiao et al., 1996; Irizarry et al., 1997; Frautshcy et al., 1998; Kawarabayashi et al., 2001; Le et al., 2001; Tomidokoro et al., 2001). β-amyloid plaques in Tg2576 mice are associated with tau- (with variable phosphorylation), PHF-tau-, NF triplet protein-, MAP2-, dephosphorylated NF triplet protein-, APP-, PS1-, synaptophysin-, α-synuclein- and ubiquitin-labelled DNs (Irizarry et al., 1997; Le et al., 2001; Tomidokoro et al., 2001; Motoi et al., 2004; Noda-Saita et al., 2004), reactive astrocytes (Hsiao et al., 1996; Irizarry et al., 1997; Motoi et al., 2004) and microglia (Frautschy et al., 1998; Sasaki et al., 2002). No neuropil threads or NFTs are present in the Tg2576 mouse model (Noda-Saita et al., 2004). Results regarding neuron loss in Tg2576 mice are contradictory. One study reports no neuronal loss in the hippocampal CA1 (Irizarry et al., 1997), yet another study detects a significant decrease of neuronal numbers in areas containing dense cored Aβ plaques compared with the corresponding areas in wild type control mice (Tomidokoro et al., 2001). Although memory deficits in Tg2576 mice were

originally reported to commence at nine months of age (Hsiao et al., 1996), more recent studies detected age-related memory deficits in Tg2576 mice at six-nine months of age, which does not correlate with the initiation of Aβ plaque deposition, but may be associated with Aβ assemblies within the brain (Kotilinek et al., 2002; Westerman et al., 2002; Arendash et al., 2004a; Jacobsen et al., 2006; Middei et al., 2006).

1.8.1.2 PDAPP and APP23 transgenic AD mice

Two comparable and widely used mouse models of AD that develop age-related AD-like pathology earlier than Tg2576 mice are the PDAPP and APP23 mouse models of AD. β-amyloid plaque deposition initiates at approximately six months of age in both PDAPP and APP23 mice (Games et al., 1995; Irizarry et al., 1997; Sturchler-Pierrat et al., 1997). In PDAPP mice Aβ plaques are present in the hippocampal formation, corpus callosum and cerebral cortex (Games et al., 1995; Masliah et al., 1996b; Reilly et al., 2003; Bussière et al., 2004), while APP23 mice exhibit Aβ plaques in the hippocampus, cerebral cortex and cerebral vasculature and also in the thalamus, olfactory nucleus and caudate putamen in older mice (Sturchler-Pierrat et al., 1997; Calhoun et al., 1998; Kuo et al., 2001; Bondolfi et al., 2002). PDAPP and APP23 mice develop Aβ plaque-associated DNs that are immunoreactive for a variety of epitopes including APP, synaptophysin, phosphorylated tau, PHF-tau and NF triplet protein (Games et al., 1995; Masliah et al., 1996b; Irizarry et al., 1997; Diez et al., 2003; Bussière et al., 2004; Schwab et al., 2004). β-amyloid plaques are also associated with astrogliosis and microgliosis in both mouse models (Games et al., 1995; Masliah et al., 1996b; Irizarry et al., 1997; Sturchler-Pierrat et al., 1997; Bornemann et al., 2001; Schwab et al., 2004). As in Tg2576 mice, no neurofibrillary pathology is observed in PDAPP or APP23 mice (Games et al., 1995; Masliah et al., 2001; Schwab et al., 2004). Neuron loss is observed in the hippocampus and neocortex of APP23 mice, and is inversely correlated with Aβ plaque load (Calhoun et al., 1998; Bondolfi et al., 2002). In contrast, no overt neuronal loss is present in the entorhinal or cingulate cortex or the hippocampus in PDAPP mice up to 18 months of age (Irizarry et al., 1997). Both PDAPP and APP23 mice develop memory deficits beginning at three months of age, prior to Aβ plaque deposition (Dodart

et al., 1999; Kelly et al., 2003; Van Dam et al., 2003; Hartman et al., 2005). However, the extent of navigation impairment in the Morris water maze does correlate with Aβ plaque load in APP23 mice (Sykova et al., 2005), and PDAPP mice show an age-dependent decrease in spontaneous object-recognition that is more severe at ages when Aβ plaque deposition is known to occur (Dodart et al., 1999).

1.8.1.3 TgCRND8 transgenic AD mice

TgCRND8 AD mice accumulate AD-associated pathology much more rapidly than Tg2576, PDAPP and APP23 mice. β-amyloid plaques are present in TgCRND8 by three months of age, Aβ plaque deposition increases with age, and dense-cored Aβ plaques and neuritic pathology are evident at five months of age (Chishti et al., 2001; Dudal et al., 2004). TgCRND8 mice exhibit Aβ plaques in the amygdala, hippocampal formation and neocortex, then the thalamus, cerebral vasculature, striatum, cerebellum and brainstem, consecutively (Chishti et al., 2001; Dudal et al., 2004). Although the rapid accumulation of pathology in this AD mouse model does not necessarily resemble the slow increase of pathology of most human AD cases, it does provide researchers with an AD mouse model that develops pathology quickly, which is ideal to fast-track pilot studies or for testing potential therapeutics. β-amyloid plaques in TgCRND8 mice are also associated with NFH-, hyperphosphorylated-tau-, synaptophysin- and ubiquitin-labelled DNs (Chishti et al., 2001), astrogliosis and microgliosis (Chishti et al., 2001; Dudal et al., 2004; Bellucci et al., 2007). Memory deficits are observed in TgCRND8 mice beginning at three months of age, and are associated with high levels of Aβ42 production and the onset of Aβ plaque deposition (Janus et al., 2000; Chishti et al., 2001, Hyde et al., 2005; Lovasic et al., 2005).

1.8.1.4 Other widely used transgenic APP AD mice

Other widely used APP-expressing AD mouse models include the APPLon, PDAPPSwe/Ind and APPSwe/Ind/Arc models (Table 1.2). Pathology first appears in APPLon mice at 10-12 months of age, including Aβ plaques and Aβ plaque-associated DNs, astrogliosis and microgliosis in the hippocampus, cortex, thalamus, external

capsule, pontine nuclei and white matter (Moechars et al., 1999). PDAPPSwe/Ind mice develop Aβ plaque pathology that begins at five to seven months of age in the dentate gyrus and neocortex, Aβ plaque pathology increases with aging, and Aβ plaque-associated DNs are also observed in adult mice (Mucke et al., 2000; Descarries et al., 2005). Finally, the relatively new APPSwe/Ind/Arc AD mouse model expresses APP harbouring the familial Swedish, Indiana and Arctic mutations (Cheng et al., 2004). The Arctic APP mutation results in Aβ more easily forming protofibrils *in vitro* (Lord et al., 2006). Thus, Aβ plaque deposition in APPSwe/Ind/Arc mice occurs earlier, at two to three months of age, and is more extensive than in APPSwe/Ind mice (Cheng et al., 2004).

1.8.2 Double transgenic mouse models of AD

Double transgenic AD mouse models generally entail mice expressing human APP harbouring mutations associated with familial AD in combination with mutant or non-mutant human PS or tau.

1.8.2.1 PS/APP double transgenic AD mice

There are many APP and PS1 or PS2 double transgenic mice, which generally develop AD-like pathology earlier and more extensively than APP transgenic mice (Holcomb et al., 1998; Chishti et al., 2001; Lee et al., 2001; Wirths et al., 2001; Blanchard et al., 2003; Richards et al., 2003; Wang et al., 2003a; Savonenko et al., 2005). In comparison, mice overexpressing only human mutant PS1, but not wild type PS1, demonstrate increased Aβ42/43, do not develop Aβ plaques and exhibit no behavioural abnormalities (Duff et al., 1996; Guo et al., 1999; Sadowski et al., 2004). However, mice expressing human mutant or wild type PS2 exhibit behavioural dysfunction and immolabelling for Aβ1-42 and human PS2 that is not present in age-matched non-transgenic control mice (Hwang et al., 2002). While other transgenic mice expressing wild type PS2 exhibit no increase in Aβ1-42 and Aβ1-40 production, but mice expressing familial AD mutant PS2 support robust production of Aβ1-42 (Mastrangelo et al., 2005).

Perhaps the most commonly used APP/PS double transgenic mouse is the PSAPP AD mouse model (Table 1.2). β-amyloid plaque deposition initiates at 10 weeks of age in PSAPP mice compared to seven months of age in Tg2576 mice, probably due to increased levels of Aβ1-40 and Aβ1-42 in PSAPP mice compared to Tg2576 mice (Holcomb et al., 1998; McGowan et al., 1999; Takeuchi et al., 2000; Howlett et al., 2004). Additionally, PSAPP mice develop larger numbers of fibrillar Aβ plaques than Tg2576 mice (Holcomb et al., 1998; Le et al., 2001). The localisation of Aβ plaques in PSAPP mice is similar to that of Tg2576 mice, with Aβ plaque deposition in the hippocampal formation, cerebral cortex, corpus callosum and cerebral vasculature (Holcomb et al., 1998; McGowan et al., 1999; Takeuchi et al., 2000; Kurt et al., 2003; Howlett et al., 2004). PSAPP mice also exhibit Aβ plaque-associated DNs (Lee et al., 2001; Kurt et al., 2003) and astrogliosis (McGowan et al., 1999), but no NFTs or overt neuronal loss in the hippocampus and cortices (Takeuchi et al., 2000; Sadowski et al., 2004). Although one study reports that PSAPP mice exhibit no impairments in spatial navigation at six to nine months of age (Holcomb et al., 1999), other studies detect memory deficits in six to 10 month old PSAPP mice (Howlett et al., 2004; Sadowski et al., 2004; Trinchese et al., 2004). These memory deficits do not match initiation of Aβ plaque deposition in PSAPP mice, but do correspond with increased Aβ plaque burden and the formation of DNs and gliosis (Howlett et al., 2004; Trinchese et al., 2004), suggesting that cognitive impairments in PSAPP mice are not simply related to Aβ deposition but may result from the disruption of neural connectivity (Howlett et al., 2004). In addition, abnormal long-term potentiation is observed in PSAPP mice at three months of age, paralleling the appearance of Aβ plaques in this mouse model (Trinchese et al., 2004).

1.8.2.2 Tau/APP double transgenic AD mice

Transgenic tau mice have been developed that overexpress various isoforms of human tau with or without mutations associated with hereditary frontotemporal dementia and parkinsonism (Lewis et al., 2000; Gotz et al., 2001; Zhang et al., 2004a; SantaCruz et al., 2005; Terwel et al., 2005). Overexpression of non-mutant human tau results in axonal

dilations and spheroids and normal survival in transgenic mice (Terwel et al., 2005), but the overexpression of mutant tau, which potentially reduced the binding of tau to microtubules, results in the development of intra-neuronal NFTs and reduction in life span (Lewis et al., 2000; Gotz et al., 2001; Zhang et al., 2004a; Terwel et al., 2005). In addition, when the expression of mutant tau is repressed in two and a half month old transgenic tau mice (rTg(tauP301L) 4510; Table 1.2) pathological progression stops, but when expression of the transgene is suppressed in mice that are four and half months of age or older, NFTs continued to accumulate (SantaCruz et al., 2005). Interestingly, in rTg(tauP301L) 4510 mice in which transgene expression is suppressed at two and half months of age, memory function improves despite ongoing accumulation of NFTs, suggesting that NFT formation does not disrupt cognitive function (SantaCruz et al., 2005). However, the cognitive deficits in rTg(tauP301L) 4510 mice are associated with the presence of oligomeric aggregates of tau, which are also present in JNPL3 mice and human AD (Berger et al., 2007).

Two APP/tau double transgenic mouse models currently exist, namely APPSw/Tau/v/w and TAPP mice (Table 1.2). APPSw/Tau/v/w mice develop enhanced Aβ plaque deposition in combination with neurofibrillary pathology and overt neuron loss in limbic areas (Ribe et al., 2005). Similarly, TAPP mice exhibit Aβ plaques at the same age as Tg2576 mice, but also produce enhanced NFT pathology and pre-NFTs in the limbic system and olfactory cortex, areas that rarely contain NFTs in JNPL3 mice (Lewis et al., 2001). The data from these two transgenic mice suggest that reciprocal interactions occur *in vivo* between APP or Aβ and tau (Lewis et al., 2001; Ribe et al., 2005).

1.8.3 Triple transgenic AD mice

Finally, the 3xTg-AD mouse model combines the expression of mutated human APP, PS1 and tau (Table 1.2). 3xTg-AD mice exhibit Aβ plaque and neurofibrillary pathology in a very similar regional and temporal pattern to human AD (Oddo et al., 2003a). Tau pathology begins in the hippocampus of 3xTg-AD mice and progresses into the cortex, whereas Aβ plaques are first observed in the cortex and then in the

hippocampus (Oddo et al., 2003b). A subset of Aβ plaques in 3xTg-AD mice are also associated with astrogliosis (Oddo et al., 2003b) and tau-labelled DNs (Oddo et al., 2003a). Homozygous 3xTg-AD mice first develop Aβ plaques at six months of age, prior to the presence of NFTs despite equivalent expression of both transgenes (Oddo et al., 2003a; Oddo et al., 2003b). In addition, increasing the tau expression levels in 3xTg-AD mice has no effect on the initiation or progression of Aβ expression or deposition in these mice (Oddo et al., 2007). Thus, 3xTg-AD mice provide further support for the amyloid cascade hypothesis (Oddo et al., 2003b; Oddo et al., 2007). A memory deficit can be detected in 3xTg-AD mice at four months of age, a time point at which Aβ plaques and NFTs are not observed, but intraneuronal Aβ is present in the hippocampus and amygdala (Billings et al., 2005). Furthermore, this cognitive deficit is rescued when intraneuronal Aβ is cleared by immunotherapy, suggesting that intraneuronal Aβ may also have a role in the inception of cognitive dysfunction (Billings et al., 2005). However, a further study found that immunisation protocols that reduce soluble Aβ and tau ameliorate cognitive deficits in 3xTg-AD mice, but immunisation that reduces soluble Aβ alone does not (Oddo et al., 2006).

1.9 THERAPEUTIC INTERVENTIONS FOR AD

There are presently no effective treatments to slow down the progression or to decrease the symptoms of AD (Parnetti et al., 1997). Currently, the therapies most commonly available for AD patients are cholinesterase inhibitors, which enhance cholinergic neurotransmission (Brion, 1996; Brodaty et al., 2001) and N-methyl-D-aspartate (NMDA) glutamate receptor antagonists (Livingston and Katona, 2004). However, current research suggests that some cholinesterase inhibitors may have other pharmacological modes of action. For example, Huperzine A is a potent acetyl-cholinesterase inhibitor that is in Phase II clinical trials in China and the United States, which may also protect neurons by upregulating nerve growth factor (NGF), binding to NMDA receptors, interfering with APP processing to increase soluble APP levels, reducing glutamate-induced excitotoxicity and improving long-term potentiation, (Gordon et al., 2001; Jiang et al., 2003; Zhang et al., 2004b; Wang et al., 2006).

However, the currently available cholinesterase inhibitors and NMDA antagonist therapeutics for AD only decrease the clinical manifestation of AD in subset of patients, and this reprieve is usually only temporary, as these therapeutics do not affect disease progression (Parnetti et al., 1997). Thus, there is dire need for therapeutic interventions that either slow or stop the progression of AD.

Assuming that Aβ plaques are the primary etiological agent of AD, two possible approaches to treating AD exist: to prevent Aβ plaque formation or to protect neurons against the damaging effects of Aβ (Vickers et al., 2000). Therapeutics that aim to protect neurons from the damaging effects of AD pathology include anti-inflammatory drugs, dietary supplements and antioxidants, growth factor therapy and cytoskeletal stabilising drugs. While therapeutic interventions with the goal of preventing or reducing Aβ levels and Aβ plaque formation include drugs that modulate APP processing, Aβ aggregation inhibitors, and therapeutics that increase Aβ clearance or degradation. The following part of this review highlights the many avenues of research into discovering and developing new therapeutics for AD that slow or halt disease progression.

1.9.1 Anti-inflammatory drugs

Anti-inflammatory drugs initially became the focus of AD research due to epidemiology studies, which suggest that the use of anti-inflammatory drugs reduced the risk of developing AD. Indeed, recent research does suggest that several nonsteroidal anti-inflammatory medications (NSAIDs) lower Aβ levels in cultured cells and the brains of Tg2576 mice, by targeting the γ-secretase complex that cleaves APP (Weggen et al., 2001; Eriksen et al., 2003). However, studies assessing the effectiveness of anti-inflammatory treatment of AD in humans, mostly concerning NSAIDs or NSAID related compounds, failed to show any benefit to the subjects in the study (as reviewed in Rozemuller et al., 2005). In addition, a large trail of NSAIDs (the AD anti-inflammatory prevention trial) was undertaken by the United States National Institute on Aging, but was halted in 2004, as there was evidence of an increased risk of

cardiovascular events in the NSAID treatment group (Lyketsos et al., 2007). Furthermore, initial follow up studies of the AD anti-inflammatory prevention trial does not show that NSAIDs prevented AD (Lyketsos et al., 2007).

1.9.2 Dietary supplements and antioxidants

There is increasing evidence to suggest that diet may affect the risk of developing AD (Engelhart et al., 2002; Luchsinger et al., 2002; Morris et al., 2002; Luchsinger et al., 2003; Luchsinger et al., 2007). In particular, foods high in antioxidants may slow the progression of AD by combating the effects of damaging free radicals (Markesbery and Carney, 1999; Engelhart et al., 2002). For example, there is evidence that the antioxidants Ginkgo biloba, curcumin, resveratrol, polyphenols and vitamin E all reduce Aβ pathology or Aβ plaque-associated oxidative stress in transgenic AD mouse models (Sung et al., 2004; Marambaud et al., 2005; Rezai-Zadeh et al., 2005; Garcia-Alloza et al., 2006; Hartman et al., 2006). Specifically, pomegranate juice, which contains high levels of polyphenols, significantly decreases soluble Aβ levels and Aβ plaque loads and improves behaviour in Tg2576 AD mice (Hartman et al., 2006). Similarly, green tea pholephenoic flavonoid (-)-epigallocatechin-3-gallate also decreases Aβ levels and Aβ plaque deposition in Tg2576 mice in association with the promotion of the α-secretase APP proteolytic pathway (Rezai-Zadeh et al., 2005). However, resveratrol, strongly reduced Aβ levels in cell lines expressing wild type or Swedish mutant APP by promoting Aβ degradation via the proteasome (Marambaud et al., 2005). Both Ginkgo biloba and vitamin E also reduce the Aβ plaque-associated oxidative stress *in vivo* in double APP/PS1 transgenic mice (Garcia-Alloza et al., 2006). Interestingly, Aβ plaque size is not significantly changed by Ginkgo biloba or vitamin E, but the curvature of Aβ plaque-associated DNs is significantly reduced in Ginkgo biloba treated transgenic AD mice and shows a similar, but not significant, trend in the vitamin E treated transgenic AD mice (Garcia-Alloza et al., 2006). Furthermore, chronic dietary supplementation with vitamin E reduces Aβ deposits in Tg2576 mice (Sung et al., 2004). In human epidemiology studies vitamin E from food and vitamin E supplements are both

correlated with lower incidence of Alzheimer's disease (Morris et al., 2002; Morris et al., 2005).

In addition, supplementing diets with nutrients essential for efficient neuronal functioning are also being investigated. One such nutrient is docosahexaenoic acid (DHA), which is an omega-3 polyunsaturated fatty acid that is a major constituent of synaptic plasma membranes and has roles in membrane flexibility, signal transduction and neurotransmission (as reviewed in Horrocks and Farooqui, 2004; Marszalek and Lodish, 2005). Epidemiological studies have suggested that people with high plasma phosphatidylcholine DHA levels have a significantly reduced risk of developing dementia (Schaefer et al., 2006). Additionally, DHA supplementation in Tg2576 and 3xTg-AD mice significantly reduces Aβ levels and Aβ pathology, and corrects synaptic deficits and cognitive function (Lim et al., 2005; Cole and Frautschy, 2006; Green et al., 2007), which is attributed to decreases in presenilin 1 levels and not alterations in α- or β-secretase activity (Green et al., 2007). In addition, a clinical trial of omega-3 fatty acid in 174 mild to moderate AD subjects shows a significant reduction in cognitive decline in a subset of 32 subjects with very mild cognitive dysfunction, but no difference in the moderate AD subjects (Freund-Levi et al., 2006).

1.9.3 Growth factor treatment

The potential for NGF and brain derived neurotrophic factor (BDNF) to prevent or slow nerve cell loss in AD and other neurodegenerative diseases has been investigated for several decades. The therapeutic delivery of NGF and BDNF is problematic as neither crosses the blood brain barrier (BBB) and broad growth factor application to the brain has adverse effects, however, pharmacologically increasing endogenous growth factor expression, or administration of growth factors locally through autologous cells modified to produce growth factors, or by viral vectors are providing promising results (as reviewed in Fumgalli et al., 2006; Tuszynski, 2007). Although it should be noted that even if growth factors can be successfully administered to AD subjects it is unlikely

that growth factor therapy will "cure" AD, as so far there is no evidence that growth factors are able to slow the progression of AD (Tuszynski et al., 2005; Tuszynski, 2007). A recent Phase I trial of implanting autologous fibroblasts, genetically modified with a retroviral vector to express human NGF, into the forebrain of eight mild AD subjects shows that such therapy may be viable (Tuszynski et al., 2005). No long-term adverse reactions to the fibroblast implantation were present in the follow up of six subjects (Tuszynski et al., 2005). Cognitive function results are also promising, with three of the six subjects showing improved or stable cognitive function and two subjects showing decreased cognitive decline (Tuszynski et al., 2005). In addition, a robust cholinergic axonal sprouting response is observed into the site of NGF delivery, and the establishment of new connections may at least partially explain the positive changes observed in cognition (Tuszynski et al., 2005).

Other therapeutics are also being investigated that induce the endogenous expression of growth factors (eg. Neotrofin, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor modulators), or mimic growth factor activities (eg. Cerebrolysin). For example, Neotrofin (AIT-082, NeoTherapeutics Inc.) activates a second messenger system that results in the production of mRNA for neurotrophins. Phase I trials with Neotrofin are complete (Grundman et al., 2003), and Neotrofin is now in Phase II/III clinical trials. The advantage of Neotrofin is that it is orally active, penetrates the blood-brain barrier and induces the production of multiple growth factors within the CNS. Furthermore, cerebrolysin (Ebewe Pharmaceutical) is an injectable neuroprotective, neurotrophic protein with NFG-like activity that has undergone human clinical trials (Ruther et al., 2000; Panisset et al., 2002; Ruether et al., 2002; Alvarez et al., 2006). In addition to its neuroprotective and neurotrophic ability, cerebrolysin decreases the production of full length APP, and C terminal APP fragments probably by regulating the maturation of the APP and its transport to cellular sites where Aβ is generated (Rockenstein et al., 2006). Thus, cerebrolysin treatment in transgenic AD mice results in decreased Aβ plaque deposition and synaptic pathology, and ameliorates learning and memory deficits (Rockenstein et al., 2003; Rockenstein et al., 2006). Finally, human clinical trials show that cerebrolysin improves cognitive and behavioural outcomes in humans AD subjects,

which are still detectable up to six months after a four week treatment course of cerebrolysin (Ruther et al., 2000; Panisett et al., 2002; Ruether et al., 2002, Alvarez et al., 2006).

1.9.4 Cytoskeletal stabilising drugs

The hyperphosphorylated tau that is present in NFTs, pre-NFTs, DNs and neuropil threads in AD, is less able to bind to and stabilise microtubules. Thus, several microtubule stabilising drugs, aimed to substitute for the tau sequestered into the inclusions in tauopathies (Zhang et al., 2004c), are being investigated for use as potential AD therapeutics. Paclitaxel (Taxol®, Bristol-Myers Squibb Company) is a pro-apoptotic Bcl-2 binding and microtubule stabilising drug that is widely used to treat cancer that can protect neurons against various toxic stimuli including Aβ peptides and reactive oxygen species (Rodi et al., 1999; Michaelis et al., 1998; Sponne et al., 2003). However, Paclitaxel cannot cross the BBB so a search for novel microtubule stabilising drugs with neuroprotective properties that could also cross the BBB was undertaken (Michaelis, 2006). Thus, Taxotere, UK 100, GS164 and Tx67 were discovered, which all also significantly increase the percentage of surviving neurons following treatment with aggregated Aβ (Michaelis et al., 2002). The effects of two of these novel taxanes, namely Tx67 and KU-237, in transgenic tau mice are currently being investigated as potential therapeutics for AD (Michaelis, 2006). In addition, Paxceed™ (Angiotech Pharmaceuticals, Inc.), which is Paclitaxel in a micelle vehicle, was also developed. Tau transgenic mice treated with Paxceed™ exhibit ameliorated motor impairments compared to sham treated mice, which is associated with restored fast axonal transport and increased numbers of microtubules and stable tubulins (Zhang et al., 2004c). These results support a potential therapeutic role for microtubule stabilising drugs in the treatment of neurodegenerative tauopathies (Zhang et al., 2004c).

1.9.5 β-amyloid precursor protein processing modifying drugs

Therapy aimed to modify APP processing to produce less Aβ includes β-secretase and γ-secretase inhibitors, statins, heparins, and antibodies against the β-secretase cleavage site of APP, as well therapeutics that increase Aβ clearance through the upregulation of Aβ degrading enzymes.

Inhibition of β-secretase and γ-secretase reduces the production of Aβ, with the aim of stopping or slowing the progression of Aβ deposition and/or toxicity. Few human clinical trials have been undertaken for β-secretase and γ-secretase inhibitors so far. However, experiments in which PDAPP mice are crossed with APP-cleaving β-secretase BACE-1 knock out mice provide proof of concept, as PDAPP/BACE-$1^{-/-}$ mice do not produce Aβ, Aβ plaques or have synaptic deficits (McConlogue et al., 2007). In addition, PDAPP/BACE-$1^{+/-}$ mice initially exhibit only a 12% decrease in Aβ levels, but this is enough of a reduction to result in a dramatic decrease in Aβ plaque deposition, DN burden and synaptic deficits in the aged mice (McConlogue et al., 2007).

GSK188909 is a relatively new selective peptide BACE-1 inhibitor, which inhibits β-cleavage of APP and reduces secreted and intracellular Aβ in an APP expressing cell line (Hussain et al., 2007). Additionally, GSK188909 can lower brain Aβ levels following oral administration in APP/PS1 transgenic AD mice (Hussain et al., 2007), but no clinical trials for GSK188909 are underway as yet. In contrast, Ly450139 dihydrate is a γ-secretase inhibitor that is currently in Phase II clinical trials. Although initial results in transgenic PDAPP mice show a reduction of Aβ levels and Aβ plaque burden (Ness et al., 2004 presented at the 8th International Montreal/Springfield Symposium on Advances in Alzheimer Therapy), human clinical trials report that plasma Aβ is only lowered for six hours following Ly450139 dihydrate administration in human subjects, and that cerebrospinal fluid Aβ is unchanged (Siemers et al., 2005; Siemers et al., 2006). Another γ-secretase inhibitor that is in human clinical trials is Flurizan™ (r-flurbiprofen). Flurizan™ does not inhibit cyclooxygenase, but does lower Aβ1-42 levels and Aβ plaque deposition by selectively modulating γ-secretase activity (Eriksen et al., 2003). In transgenic AD mice Flurizan™ reduces Aβ levels, and Aβ plaque load and

results in decreased memory and learning deficits (Kukar et al., 2007), and in human trials Flurizan™ significantly improves cognitive function in mild AD subjects (Wilcock et al., 2005 presented at Alzheimer's Association International Conference on Prevention of Dementia). Two Phase III clinical trials for Flurizan™ are now underway. Finally, ongoing research aimed at discovering or designing new more efficient secretase inhibitors is also providing some promising results in transgenic AD mice (Best et al., 2007; Prasad et al., 2007).

Statins are HMG Co-A reductase inhibitors that decrease de novo cholesterol synthesis, and are currently used to lower elevated low-density lipoprotein cholesterol levels. Epidemiology studies suggest that statins may also reduce the risk of dementia (Jick et al., 2000). Thus, several statins are currently in Phase II/III clinical trials for AD including atorvastatin, verivastatin, fluvastatin, lovastatin, pravastatin and simvastatin, and are thought to act by altering APP metabolism to reduce the production of Aβ (Simons et al., 2002; Hoglund et al. 2005; Sparks et al., 2005). For example, simvastatin is hydrolysed *in vivo* to produce a metabolite that competes with HMG-CoA, which is a rate limiting enzyme for cholesterol synthesis, and simvastatin also inhibits both α- and β-secretase in the brain (Simons et al., 2002; Sjogren et al., 2003). However, Tg2576 mice treated with simvastatin exhibit improvements in learning and memory, but no changes in the level of Aβ, or Aβ plaques within their brains (Li et al., 2006). In addition, treating 19 human AD subjects with doses of simvastatin, which affects cholesterol metabolism in the CNS, for 12 months, did not significantly change cerebrospinal fluid Aβ levels or cognition compared to untreated controls subjects (Hoglund et al. 2005). While a shorter term simvastatin treatment trial in human AD patients found no significant decrease in cerebrospinal fluid Aβ levels overall, a subset of subjects with mild AD demonstrate a significant reduction in cerebrospinal fluid Aβ1-40 levels (Simons et al., 2002). Similarly, heparins are generally utilised as anti-coagulant agents, but heparins and related heparin oligosaccharides also inhibit proteoglycan assembly and have anti-inflammatory properties that may have beneficial effects in AD subjects (Ma et al., 2007b). For example, enozaparin, a low molecular weight heparin, significantly reduces Aβ plaque load, and Aβ1-40 levels and the number

of activated astrocytes adjacent to Aβ plaques in transgenic AD mice (Bergamaschini et al., 2004). Finally, lithium is another therapeutic agent, commonly used for treatment of bipolar disorder, which is also currently under investigation for use as an AD therapeutic. Lithium reduces the activity of GSK3, which plays a role in tau phosphorylation and the regulation of APP processing (Engel et al., 2006; Caccamo et al., 2007; Rockenstein et al., 2007). Transgenic AD mice expressing mutated APP treated with lithium chloride display reduced GSK3β activity, APP phosphorylation and Aβ production, improved learning and memory, preserved dendritic structure and decreased tau hyperphosphorylation (Rockenstein et al., 2007). This study also suggests that lithium chloride may exert some of these positive effects by reducing GSK3β activity, which in turn modulates the processing of APP (Rockenstein et al., 2007). However, lithium administration to 3xTg-AD mice reduces tau phosphorylation, but does not reduce Aβ plaque loads or improve memory function (Caccamo et al., 2007). In addition, early lithium administration to a tau-only transgenic mouse model can prevent NFT formation, but lithium treatment cannot alter existing NFTs (Engel et al., 2006).

1.9.6 Aggregation inhibitors

Many approaches to Aβ plaque prevention or removal are under investigation (Vickers et al., 2000; Ono et al., 2006). In fact, two Aβ aggregation inhibitors, AZD-103 (Transition Therapeutics) and Alzhemed™ (Neurochem Inc.), are currently in Phase 1 and Phase 3 human trials, respectively. Alzhemed™ is an orally bioavailable organic molecule that interferes with the association between glycosaminoglycans and Aβ, can prevent the formation and deposition of fibrillar Aβ and also binds to soluble Aβ.

Other Aβ aggregation inhibitors are copper and zinc chelators. β-amyloid has both high and low affinity copper and zinc binding sites that affect its reversible precipitation into insoluble masses (Bush et al., 1994; Huang et al., 1997; Atwood et al., 1998) and the production of hydrogen peroxide that is mediated by redox-active metal ion and Aβ interactions (Cherny et al., 1999; Huang et al., 1999; Opazo et al., 2002). Two copper

and zinc chelators that inhibit zinc and copper induced Aβ aggregation are now in clinical trails (Ritchie et al., 2003; PranaBiotechnology Limited). One such metal chelator is clioquinol (5-chloro-7-iodo-8-hydroxyquinoline), which significantly decreases Aβ plaque deposition in Tg2576 mice (Cherny et al., 2001). In addition, a small pilot Phase II clinical trial of clioquinol showed decreases in plasma Aβ1-42 levels in the clioquinol treatment group compared to the placebo treated group, but a significant improvement in cognitive decline was only detected in severe AD subjects treated with clioquinol compared to severe AD placebo treated subjects (Ritchie et al., 2003). Another metal chelator that is also currently in Phase II clinical trials is PBT2 (Prana Biotechnology Ltd.). PBT2 is a small orally effective molecule that was designed to inhibit catalytic redox reactions by preventing Aβ from abnormally binding to copper, and the subsequent generation of hydrogen peroxide. Thus, PBT2 inhibits the redox-dependent formation of oligomeric Aβ, prevents Aβ plaque deposition, improves cognitive performance in Tg2576 and APP/PS1 mice and may promote normal copper and zinc homeostasis within the brain (Adlard et al., 2007 presented at the International Brain Research Organisation World Congress of Neuroscience; Finkelstein et al., 2007 presented at the International Brain Research Organisation World Congress of Neuroscience).

However, as other *in vitro* and *in vivo* studies (Lambert et al., 1998; Hartley et al., 1999; Huang et al., 2006; Chauhan, 2007; Lacor et al., 2007; Matsayama et al., 2007) suggest that it is soluble Aβ assemblies that are toxic and cause behavioural deficits in AD, accelerating Aβ fibrilisation may be an alternative therapeutic intervention. Recent research suggests that increasing Aβ fibrilisation reduces soluble Aβ levels and behavioural impairments in transgenic mice expressing human APP harbouring the Arctic mutation (Cheng et al., 2007). Additionally, if soluble Aβ is the toxic species of Aβ, therapeutics that disaggregate and solubilise Aβ plaques on a large scale may have detrimental effects. For example, AD subjects in the clinical AN-1792 immunisation trial exhibit decreased parenchymal Aβ plaques, but increases in soluble Aβ levels and vascular Aβ deposits compared to non-immunised AD subjects, suggesting that

immunisation disrupts Aβ plaques but vascular capture of Aβ prevented large scale removal of soluble Aβ from the brain (Patton et al., 2006).

1.9.7 Therapeutics that increase the clearance of Aβ

Interest in immunotherapy for AD was ignited in 1999 when a paper by Schenk and colleagues was published, reporting the success of an active immunisation protocol in a mouse model of AD. In this study PDAPP mice were immunised with Aβ1-42 and adjuvant at 11 months of age, when neuropathology is well established, resulting in a significant reduction in Aβ burden, astrogliosis and neuritic pathology (Schenk et al., 1999). A reduction of Aβ levels, clearance or prevention of Aβ plaques and associated neuritic dystrophy, astrogliosis and microgliosis have been replicated with varying degrees of success in many different AD mouse models (Table 1.2) using both active (Schenk et al., 1999; Janus et al., 2000; Sigurdsson et al., 2001; Bard et al., 2003; Das et al., 2003; Lemere et al., 2003; Zhang et al., 2003; Hara et al., 2004; Kim et al., 2004; Schultz et al., 2004; Bowers et al., 2005; Buttini et al., 2005; Frenkel et al., 2005) and passive (Bard et al., 2000; Bacskai et al., 2002; Chauhan and Siegel, 2003; Lombardo et al., 2003; Wilcock et al., 2003; Bussière et al., 2004; Horikoshi et al., 2004; Oddo et al., 2004; Wilcock et al., 2004; Brendza et al., 2005; Chauhan and Siegel, 2005; Hartman et al., 2005; Yamamoto et al., 2005; Levites et al., 2006) immunisation protocols. The memory and learning deficits associated with many transgenic mouse models of AD are also ameliorated by immunotherapy (Janus et al., 2000; Morgan et al., 2001; Dodart et al., 2002; Kotilinek et al., 2002; Wilcock et al., 2004; Billings et al., 2005; Hartman et al., 2005; Jensen et al., 2005; Lee et al., 2006). Interestingly, these documented improvements in functional memory may be due to the recovery of cellular dynamics and normal morphology in DNs following Aβ plaque clearance (Lombardo et al., 2003; Oddo et al., 2004; Bussière et al., 2004; Brendza et al., 2005).

The success of Aβ immunisation in terms of reduced Aβ plaque burden, neurite dystrophism, reactive astrogliosis and microgliosis and positive functional outcomes in many different mouse models of AD captured corporate interest and Elan Corporation

and collaborators Wyeth-Ayerst initiated human trials with AN1792, an aggregated human Aβ1-42 peptide, with QS-21 adjuvant. Although Phase 1 trials reported no ill effects of active immunisation with AN1792 (Aβ1-42 and QS-21 adjuvant) (Check, 2002), the Phase 2A trial for therapy effectiveness was terminated when six percent of patients (18 of 298 treated with AN1792) developed aseptic meningoencephalitis (Orgogozo et al., 2003; Gilman et al., 2005).

Despite this adverse reaction to active immunisation with Aβ in the human population, post-mortem analysis of three subjects from the AN1792 trial (two suffered meningoencephalitis) reveals widespread clearance of Aβ plaques and astrocyte clusters, variable clearance of tau-labelled DNs and amyloid angiopathy, but no reduction of NFTs or neuropil threads (Nicoll et al., 2003; Ferrer et al., 2004; Nicoll et al., 2006 Bombois et al., 2007). Long-term follow-up studies on the AN1792 trial also show significantly lower cognitive decline and significantly higher quality of life scores in antibody responders (Hock et al., 2003; Gilman et al., 2005). Thus, interest in alternate active and passive immunisation regimes continues.

The time-course of immunisation, route of administration and the antigen or antibody used for immunisation will all affect the immunological outcome. Interest in passive immunisation regimes continue with clinical trials currently in progress for several new therapeutic protocols including a 3D6-based antibody (Elan Pharmaceuticals/Wyeth), LY206430 the humanised version of m266 antibody that recognises Aβ16-23 (Eli Lilly and Co.) and IVIg, a mixture of purified polyclonal antibodies from blood donors (Baxter Bioscience; Dodel et al., 2004). Investigations into utilising shorter Aβ peptide immunogens or DNA encoding Aβ as an active immunogen are already underway and show promising results (Qu et al., 2004; Schultz et al., 2004; He et al., 2005; Maier et al., 2006; Okura et al., 2006). Additionally, immunising transgenic AD mice with viral vectors expressing Aβ peptides successfully decreases levels of Aβ plaque deposition in the brain and improves cognition function (Zhang et al., 2003; Hara et al., 2004; Kim et al., 2004; Bowers et al., 2005). Active immunotherapies including CAD106 (Novartis/Cytos Biotechnology) and ACC-001 (Elan Pharmaceuticals/Wyeth) are also

currently undergoing human trials. CAD106 consists of the first six N-terminal amino acids of Aβ attached to a virus-like particle. Similarly, ACC-001 consists of an Aβ fragment attached to a conjugate carrier protein to aid the induction of an antibody response to Aβ. Immunotherapy undoubtedly results in the clearance of cerebral Aβ plaques and reductions in the Aβ plaque-associated neuritic dystrophy, astrogliosis, microgliosis and improved cognitive performance in transgenic mouse models of AD. However, the conversion of immunotherapy protocols that are effective in mice for human use may not be as simple as initially hoped. Therefore, it is important to keep exploring alternative interventions as AD therapeutics.

Finally, therapeutics other than immunisation protocols that increase the clearance of Aβ are also being investigated (Leissring et al., 2003; Risner et al., 2006; Riddell et al., 2007). Transgenic APP mice that also overexpress insulin-degrading enzyme or neprilysin, both of which degrade Aβ, exhibit reduced Aβ levels and Aβ plaque deposition (Leissring et al., 2003), providing proof of concept for therapeutics aimed at increasing endogenous enzymatic Aβ degradation. One such agent is TO901317 is a liver X receptor agonist that is being investigated for its potential to increase Aβ clearance through the induction of genes involved in intracellular lipid transport and efflux (Riddell et al., 2007). TO901317 treatment decreases Aβ1-42 levels and memory deficits in Tg2576 mice, with no effects on the levels of full length APP, Aβ1-40 or other APP processing products, suggesting that TO901317 specifically increases Aβ1-42 clearance (Riddell et al., 2007). Finally, as there is an increased risk of developing AD in type 2 diabetes patients (Arvanitakis et al., 2004), and diet-induced insulin resistance in Tg2576 mice decreases insulin-degrading enzyme activity, increases γ-secretase activity, memory deficits, Aβ levels and plaque load (Ho et al., 2004), rosiglitazone maleate, a nuclear hormone receptor peroxisome proliferator-activated receptor gamma agonist that is usually used to reduce blood glucose levels and hyperinsulineamia in diabetics, is currently in Phase III trails for use in AD subjects (GlaxoSmithKline). Rosaglitazone treatment in Tg2576 mice improves learning and memory, ameliorates reduced insulin-degrading enzyme activity, and decreases brain Aβ levels without affecting Aβ plaque load (Pedersen et al., 2006). In humans rosaglitazone improves

cognition in a subset of human mild-moderate AD subjects that are apoε4 allele carriers (Risner et al., 2006).

A better understanding of the link between Aβ deposition and the neuronal pathology apparent in AD will lead to more effective treatment of the disease. Reducing the classic reactive changes of injured neurons, or inhibiting or encouraging the resolution of the regenerative responses exhibited by injured neurons may also be an approach that will reduce neurodegeneration in AD. However, successful application of such therapeutics in clinical settings would need to occur early in the disease process before substantial neuronal degeneration has occurred. The accurate diagnosis of preclinical AD cases prior to the clinical diagnosis of AD utilising a combined biomarker/imaging approach may soon be possible (as reviewed in Borroni et al., 2007; de Leon et al., 2007), due to improved technology, imaging techniques, the discovery of biomarkers, and the development of new labels for Aβ and tau that can be used *in vivo* (Shoghi-Jadid et al., 2002; Klunk et al., 2004; Mintun et al., 2006; Small et al., 2006; Klunk et al., 2007; Wierenga and Bondi, 2007).

1.10 PROJECT AIMS

While the pathological hallmarks of AD and cellular pathways involved in the production, processing and functions of Aβ and tau have been extensively investigated, the primary causative agent or mechanisms of AD are still unknown. In addition, the exact cellular pathways that link the development of Aβ plaques, DNs, NFTs and neuropil threads to one another and to the neurodegeneration and neuronal loss characteristic of AD have not yet been elucidated. Examining the cellular and biochemical mechanisms that underlie the initiation and progression of AD is vital, not only for a complete understanding of the disease process, but also for the identification of targets for the development of effective treatments to stop or slow AD progression. Therefore, this thesis will investigate several aspects of the pathological changes leading to neuronal degeneration in AD.

Aim 1

To investigate the role of apoptosis in AD

To date, the literature regarding the role of apoptosis in AD provides evidence both for and against apoptosis playing a major role in the progression and neurodegeneration that occurs in AD. Therefore, to clarify this contentious area of AD research, apoptotic nuclear morphology and a comprehensive set of apoptotic-related proteins will be examined via immunohistochemistry in human AD, preclinical AD and control cases. In addition, the levels of mRNA for apoptosis-related proteins will also be quantified in the same cohorts of cases.

Aim 2

To examine the relationship between cytochrome c release and the pathological hallmarks of AD

The release of cyto c from the mitochondria into the cytoplasm is indicative of the activation of apoptosis and/or mitochondrial damage or dysfunction. Although, cyto c has been previously reported to be present in a subset of DNs, the association of cyto c with the pathological hallmarks of AD, and its cellular localisation in this regard,

remains to be determined. Utilising immunohistochemical techniques the relationship between cyto c, including its cellular localisation, with Aβ plaques, DNs and NFTs will be assessed. In addition, cyto c localisation in relation to the staging of AD pathology will also be investigated.

Aim 3

To determine whether the Aβ plaque-associated neuronal pathology in two transgenic AD mouse models more closely resembles that of human preclinical AD or AD cases

The Aβ plaque-associated neuronal pathology in human preclinical AD cases differs from that in AD cases, suggesting that this neuronal pathology matures or develops as AD progresses. These experiments will seek to assess whether the Aβ plaque-associated neuronal pathology in two widely utilised transgenic AD mouse models, namely TgCRND8 and Tg2576 mice, mimic the pathology present in human preclinical AD or AD cases. Utilising immunohistochemistry, the percentage of neuritic Aβ plaques, the morphology of Aβ plaque-associated dendrites and the morphology and neurochemistry of DNs will be compared to that present in human preclinical AD and AD cases.

Aim 4

To assess metallothionein isoform IIA as a potential therapeutic agent for AD utilising a mouse model of AD

The final aim of this thesis is to investigate the potential of metallothionein isoform IIA (MTIIA) as a therapeutic intervention of AD. Currently available therapeutics for the treatment of AD only address the disease's symptoms and do not affect disease progression. Thus, research into new therapeutic agents with potential modes of action that will inhibit or halt AD pathology is imperative. Metallothionein IIA is a small protein with neuroprotective, metal chelating and antioxidant properties that has previously improved outcomes in experimental models of CNS injury, multiple sclerosis and Parkinson's disease. In these experiments MTIIA or saline solution will be administered to Tg2576 mice and the Aβ-labelled and thioflavine s-stained plaque loads and Aβ plaque-associated neuronal pathology will subsequently be assessed.

2 MATERIALS AND METHODS

2.1 HUMAN BRAIN TISSUE SOURCES AND PROCESSING

Human brain tissue was obtained from multiple sources: National Tissue Resource Centre, University of Melbourne (Australia), Sun Health Research Institute (Arizona, USA), National Health and Medical Research Council Brain Bank (Adelaide, Australia) and the Department of Pathology, University of Sydney (Australia), as previously described (Saunders et al., 1998; Dickson et al., 1999). Permission for brain autopsy and use for research were obtained by the original tissue sources, and the Tasmanian Human Research Ethics Committee approved all research. Blocks of cerebral cortex were immersion-fixed in paraformaldehyde or 10% buffered formalin or the brains were perfusion-fixed with either 2% picric acid or 4% paraformaldehyde. Blocks of cerebral cortex were cryoprotected in 18.0%, then 30.0% sucrose solution, embedded in Shandon cryomatrix tissue compound (Thermo Scientific, Runcorn, UK) and 40 micron sections of SFG and ITG were sectioned on a cryostat.

Throughout this thesis a total of 20 AD cases, 11 preclinical AD cases and nine control cases were analysed (Table 2.1). No AD associated pathology was present in the control cases, which were of similar ages to AD and preclinical AD cases. The AD cases conform to the CERAD criteria, and demonstrate Braak stages V and VI pathology (Braak and Braak, 1991; Mirra et al., 1991). A subset of non-demented cases exist that exhibit signs of pathological aging that include widespread neocortical Aβ plaques, but no 'classical' neurofibrillary pathology or overt nerve cell degeneration (Price and Morris, 1999; Vickers et al., 2000). Such pathologically aged cases correspond to Braak stage III pathology, and may represent a preclinical stage of AD. The preclinical AD cases do not conform to the CERAD criteria for the diagnosis of clinical AD, but exhibit Braak stage III pathology including widespread non-neuritic (based on thioflavin S or PHF-tau-labelling) Aβ immunolabelled plaques in the neocortex and neurofibrillary pathology in the entorhinal formation and hippocampus (Braak and Braak, 1991; Vickers et al., 1996; Saunders et al., 1998).

Table 2.1 Human brain cases utilised for immunohistochemistry and analysis.

Type	Age (years)	Gender	Postmortem interval (h)	Cortical region	Pathological diagnosis	Source	Fixation
AD	60	M	64 5	ITG	COAD	NTRC	4% Para*
AD	65	M	3	SFG, ITG	AD	NHMRC brain bank	2% picric acid*
AD	67	M	31	ITG	Respiratory failure	NTRC	4% Para*
AD	67	M	61	ITG	AD	NHMRC brain bank	2% picric acid*
AD	71	F	13	SFG, ITG	AD	NHMRC brain bank	2% picric acid*
AD	72	F	4	SFG, ITG	AD	SHRI	4% Para*
AD	73	M	6 5	SFG, ITG	AD	NHMRC brain bank	2% picric acid*
AD	73	M	35	ITG	Pneumonia	NHMRC brain bank	2% picric acid*
AD	74	F	2	SFG, ITG	Pneumonia	SHRI	4% Para*
AD	74	M	2 75	SFG	Respiratory failure, AD	SHRI	4% Para*
AD	76	F	2 6	SFG, ITG	AD	NHMRC brain bank	2% picric acid*
AD	79	M	24	ITG	Respiratory failure	NTRC	4% Para*
AD	81	M	23 5	ITG	Cardiac failure	NTRC	4% Para*
AD	83	M	2 83	SFG, ITG	Dementia	SHRI	4% Para*
AD	83	F	5	ITG	AD	NHMRC brain bank	2% picric acid*
AD	84	F	3	SFG, ITG	AD	SHRI	4% Para*
AD	84	F	16 5	ITG	Cardiac failure	NHMRC brain bank	2% picric acid*
AD	88	M	7	SFG, ITG	Dementia	SHRI	4% Para*
AD	91	F	64	ITG	Malignant mesothelioma	NTRC	4% Para*
AD	92	F	2 25	ITG	Pneumonia	SHRI	4% Para*
Preclinical AD	71	M	32 5	SFG	Cardiac arrhythmia	Uni of Sydney	15% formalin*
Preclinical AD	74	M	31 5	ITG	Cardiac failure	NTRC	4% Para*
Preclinical AD	74	M	68	ITG	Cardiac failure	NTRC	4% Para*

Type	Age (years)	Gender	Postmortem interval (h)	Cortical region	Pathological diagnosis	Source	Fixation
Preclinical AD	78	M	2 25	SFG	Postoperative	SHRI	4% Para*
Preclinical AD	81	F	3	SFG	Cardiac arrest	SHRI	4% Para*
Preclinical AD	82	M	48 5	ITG	Cardiac infarction	NTRC	4% Para*
Preclinical AD	82	M	50	ITG	Cardiac failure	NTRC	4% Para*
Preclinical AD	84	M	3	SFG	Cardiopulmonary arrest	SHRI	4% Para*
Preclinical AD	90	M	2 16	SFG	Respiratory arrest	SHRI	4% Para*
Preclinical AD	91	M	3	SFG	Cardiac failure	SHRI	4% Para*
Preclinical AD	91	M	48	ITG	Renal failure	NTRC	4% Para*
Control	47	M	27 5	SFG	Cardiac infarction	Uni of Sydney	15% formalin*
Control	51	M	23 3	SFG	Pulmonary embolus	Uni of Sydney	15% formalin*
Control	58	M	27	SFG	Coronary disease	Uni of Sydney	15% formalin*
Control	58	F	30	ITG	Asthma	NTRC	4% Para*
Control	65	M	16	SFG	Cardiac infarction	Uni of Sydney	15% formalin*
Control	73	F	26 5	ITG	Pulmonary Embolism	NTRC	4% Para*
Control	77	M	53 5	ITG	Cardiac failure	NTRC	4% Para*
Control	79	M	57	ITG	Respiratory failure	NTRC	4% Para*
Control	84	M	55		Cardiac infarction	NTRC	4% Para*

NTRC, National Tissue Resource Centre, SHRI, Sun Health Research Institute, Uni of Sydney, University of Sydney, 4% para*, Immersion fixed in 4% paraformaldehyde, 2% picric acid, perfusion fixed in 2% picric acid, 10% formalin*, Immersion fixed in 10% formalin

2.2 HISTOLOGICAL STAINS

2.2.1 Thioflavine s-staining

The tissue sections were placed in 0.0125% thioflavine s (Sigma, St Louis, MO) dissolved in 60% 0.01M phosphate buffered saline (PBS) and 40% ethanol for three minutes in the dark on an orbital shaker at room temperature (RT). Following differentiation in two one minute incubations in 50:50 PBS:ethanol at RT in the dark, the tissue sections were washed three times with 0.01M PBS at RT in the dark on an orbital shaker. Thioflavine s stains a subset of plaques that contain fibrillar aggregates.

2.2.2 Nuclear yellow-staining

Tissue sections were incubated in 0.001% Nuclear Yellow (Sigma) in PBS for 30 minutes in the dark at RT, on an orbital shaker. Tissue sections were then washed three times with 0.01M PBS for 10 minutes each, at RT on an orbital shaker.

2.3 IMMUNOHISTOCHEMISTRY

2.3.1 Formic acid epitope exposure

Tissue sections were incubated in 90% formic acid (Sigma) for 20 minutes at RT on an orbital shaker. Brain sections were then washed seven times with 0.01M PBS for 10 minutes each, at RT on an orbital shaker.

2.3.2 Autofluorescence quenching

Brain sections were destained in 0.25% potassium permanganate for 20 minutes at RT on an orbital shaker and washed twice in 0.01M PBS for two minutes at RT. Brain sections were then de-labelled in 1.0% pot-metabisulphite and oxalic acid for one to two minutes. Tissue sections were then washed three times with 0.01M PBS for 10 minutes each, at RT on an orbital shaker.

2.3.3 Indirect fluorescent immunohistochemistry

Brain tissue sections were incubated in primary antibody solutions for 16-72 hours at RT or at 4°C (Table 2.2). Optimal antibody concentrations were individually determined for each antibody, and omitting primary antibodies eliminated all immunoreactivity. After three washes with 0.01M PBS for 10 minutes, each at RT on an orbital shaker, primary antibodies were exposed to Alexa Fluor goat anti-mouse/rabbit secondary antibodies for two hours at RT, on an orbital shaker in the dark (Table 2.3). All antibodies were diluted in 3.0% triton-X (Sigma) in 0.01M PBS to permeabilise the cell membranes. Tissue sections were then washed three times with 0.01M PBS for 10 minutes each at RT on an orbital shaker, and mounted onto microscope slides and coverslipped with permafluor aqueous mounting medium (Immunotech, Marseille, France).

2.3.4 Antigen retrieval

Brain sections were loaded into tissue cassettes, placed in 0.1M citrate buffer (pH 6.0) and heated on high for 10 minutes in a conventional microwave oven (LG MS-314SCE, 1000 watts). The tissue sections were then heated on high power for a further two and a half minutes and six minutes on medium power under pressure in a microwave tender cooker (Nordic ware, Minneapolis). The specimens were cooled to RT in citrate buffer before being transferred back into 0.01M PBS. Tissue sections were then removed from the tissue cassettes and washed three times with 0.01M PBS for 10 minutes each at RT on an orbital shaker.

2.3.5 Indirect immunoperoxidase immunohistochemistry

Brain sections were incubated in 1.0% hydrogen peroxide diluted in methanol for 15 minutes at RT on an orbital shaker, washed three times with 0.01M PBS for 10 minutes each, at RT on an orbital shaker, then incubated in primary antibody solutions for two hours at RT on an orbital shaker, and then overnight at 4°C. The sections were then washed three times with 0.01M PBS for 10 minutes each, at RT on an orbital shaker, and incubated in goat anti-mouse/rabbit immunoglobulin horse-radish peroxidase

Table 2.2 Primary antibodies used for immunohistochemistry.

Antibody name	Type	Immunoreactivity	Dilution	Source
anti-active caspase-3	R	Peptide from the p18 fragment of of cleaved procaspase-3	1 250/ 1 500	Promega (Madison, WI)
anti-active caspase-8	M	N-terminal region of p10 subunit of cleaved procaspase-8	1 100	Oncogene Research Products (San Diego, CA)
anti-active caspase-9	R	N-terminal region of 10kDa fragment of autoactivated procaspase-9	1 200	Biosource Int (Camarillo, CA)
anti-α-internexin	M	C-terminal of α-internexin	1 500	Chemicon (Temecula, CA)
anti-α-internexin	R	Whole α-internexin protein	1 500	Novus Biologicals (Littleton, CO)
anti-Bax	R	N-terminus of Bax	1 200	Santa Cruz Biotechnology (Santa Cruz, CA)
anti-Bcl-2	R	N-terminus of Bcl-2	1 200	Santa Cruz Biotechnology (Santa Cruz, CA)
anti-NCL-β-amyloid	M	A site on β-amyloid peptide	1 500	Novocastra (Newcastle, UK)
anti-pan-β-amyloid	R	All β-amyloid peptides	1 1000	Biosource Int (Camarillo, CA)
anti-calretnin	R	Calcium-bound and unbound conformations of calretnin	1 1000	Biosource Int (Camarillo, CA)
anti-chromogranin A	R	Large synaptic vesicles	1 500	DAKO (Carpinteria, CA)
anti-cytochrome c	M	Cytochrome c	1 500	BD Biosciences Pharmigen (Franklin Lakes, NJ)
anti-ferritin	R	Human ferritin	1 2000	DAKO (Carpinteria, CA)
anti-GFAP	M	Glial fibrillary acidic protein	1 500	Chemicon Int (Temecula, CA)
anti-MTI/II	M	MTI and MTII	1 500	DAKO (Carpinteria, CA)
anti-MAP2	M	Microtubule associated protein 2	1 250	Chemicon Int (Temecula, CA)
anti-oligodendrocyte	M	Myelin/Oligodendrocyte specific protein	1 500	Chemicon Int (Temecula, CA)
anti-SMI32	M	Dephosphorylated NFM and NFH	1 2000	Sternberger Monoclonals Inc (Lutherville, MD)
anti-SMI312	M	Phosphorylated NFM and NFH	1 3000	Sternberger Monoclonals Inc (Lutherville, MD)

Antibody name	Type	Immunoreactivity	Dilution	Source
anti-hyperphosphorylated -tau	M	Phosphorylated Ser202/Thr205, Ser202/ Ser205 or Ser205/Ser208 of PHF-tau (AT8)	1 500	Endogen (Woburn, MA)
anti-human tau	R	Phosphorylation independent tau	1 4000	DAKO (Carpinteria, CA)
anti-TRADD	M	C-terminal half of TRADD	1 250	BD Biosciences Pharmigen (Franklin Lakes, NJ)

M, mouse monoclonal antibody, R, rabbit polyclonal antibody, MT, metallothionein

Table 2.3 Fluorescent secondary antibodies utilised for indirect fluorescent immunohistochemistry.

Emission (nm)	Reactivity	Species	Dilution	Supplier
488	mouse IgG	goat	1:500	Molecular Probes (Eugene, OR)
594	mouse IgG	goat	1:500	Molecular Probes (Eugene, OR)
488	rabbit	goat	1:500	Molecular Probes (Eugene, OR)
594	rabbit	goat	1:500	Molecular Probes (Eugene, OR)

(1:200, DAKO) for one and a half hours at RT on an orbital shaker, followed by three washes with 0.01M PBS for 10 minutes each, at RT on an orbital shaker. All antibodies were diluted in 3.0% triton-X in 0.01M PBS to permeabilise the cell membranes. The Vectastain ABC Kit (Vector Laboratories, Burlingame, CA) was used for amplification via avidin/biotin according to manufacturers instructions. The primary antibody complex was visualised by a two to three minute incubation in Sigma Fast™ 3,3'-Diaminobenzidine tablet set (Sigma) diluted in Milli-Q® water. The tissue sections were washed three times with 0.01M PBS for 10 minutes each, at RT on an orbital shaker mounted and left to dry overnight at RT. The mounted sections were then incubated in Milli-Q® water for 20 minutes at RT followed by three minute incubations in 70%, 90% and 100% ethanol. After 30 minutes in xylene at RT the tissue sections were coverslipped with pertex (Medite, Burgdorf, Germany).

2.4 MICROSCOPY AND ANALYSIS

Immunolabelled specimens were examined using a Leica DM LB2 immunofluorescence microscope or a Leica DMB IRB inverted fluorescence microscope. Images were acquired using a cooled CCD Magnafire (Optronics) digital camera and Magnafire (version 1.0) software. Image analysis, such as area quantitation, was performed using NIH ImageJ (version 1.34s) software. Statistical analysis was performed in Microsoft Excel (Mac Os X) and Graphpad Prism4® software, with p values less than 0.05 (CI 95%) being considered statistically significant. Means were reported ± standard error of the mean (SEM). Graphs were prepared in Microsoft Excel or Prism (version 4.0c). Images for figures were prepared using Adobe Photoshop (version 9.0).

All solutions are provided in full in Appendix 1.

3 NO DIFFERENCE IN EXPRESSION OF APOPTOSIS-RELATED PROTEINS AND APOPTOTIC MORPHOLOGY IN CONTROL, PRECLINICAL ALZHEIMER'S DISEASE AND ALZHEIMER'S DISEASE CASES

3.1 INTRODUCTION

Apoptosis is a form of programmed cell death, which involves several pathways that result in the activation of caspases, and eventually cell death characterised by chromatin condensation, nuclear shrinkage, DNA fragmentation, cytoplasm condensation and disintegration. The search for signs of apoptosis including DNA fragmentation, frank apoptotic morphology, activated caspases, caspase cleavage products and changed levels of pro- and anti-apoptotic molecules in AD brains has produced contrasting and often conflicting results. The frequency of DNA fragmentation has been assessed in AD and control brains with reports of similar levels of DNA fragmentation in AD and control brains (Lucassen et al., 1997) or increased DNA fragmentation in AD brains (Smale et al., 1995; Cotman and Su, 1996; Lassmann, 1996; Troncoso et al., 1996; Lucassen et al., 1997; Sugaya et al., 1997; Masliah et al., 1998; Stadelmann et al., 1998). Despite these reports of increased DNA fragmentation in AD brains, classical apoptotic morphology is seldom observed or not observed at all in AD (Lassmann et al., 1995; Troncoso et al., 1996; Lucassen et al., 1997; Stadelmann et al., 1998; Jellinger and Stadelmann, 2000; Nunomura and Chiba, 2000; Raina et al., 2001; Raina et al., 2003). Such studies are further complicated by the fact that both DNA fragmentation and classical apoptotic morphology can occur independently of apoptosis (Raina et al., 2003; Tatton et al., 2003). Additionally, many studies have reported an increase in the levels and/or numbers of cells containing anti-apoptotic Bcl-2 (Satou et al., 1995; Su et al., 1996b; Kitamura et al., 1998), pro-apoptotic Bax (Nagy and Esiri, 1997; Su et al., 1997; Giannakopolous et al., 1999), active caspase-3 (Stadelmann et al., 1999; Zhao et al., 2003b), inactive and active caspase-8 (aC8, Rohn et al., 2001a; Pompl et al., 2003) and inactive and active caspase-9 (aC9, Rohn et al., 2002; Pompl et al., 2003) in AD brains compared to control brains, while others have not found increases in these proteins in

AD brains (MacGibbon et al., 1997; Nagy and Esiri, 1997; Kitamura et al., 1998; Stadelmann et al., 1998; Engidawork et al., 2001).

To investigate whether apoptosis plays a major role in the pathological process of AD the presence of apoptotic-like nuclei and a comprehensive array of apoptosis-related proteins from both the death receptor and mitochondrial apoptotic pathways were assessed. Using immunohistochemistry and real time reverse transcriptase polymerase chain reaction (RT-PCR), both the SFG and ITG of AD and control brains were analysed. In addition to control and AD cases, apoptosis-related changes were also investigated in preclinical AD cases, as such preclinical cases may provide important clues to the initiation and progression of neuronal pathology (Benzing et al., 1993; Lue et al., 1996; Vickers et al., 1996). So far, few studies have investigated apoptosis in such preclinical and early AD cases (Su et al., 1997; Gastard et al., 2003; Zhao et al., 2003a; Zhao et al., 2003b; Albrecht et al., 2007; Christie et al., 2007).

3.2 MATERIALS AND METHODS

3.2.1 Human brain tissue source and processing

Human brain tissue was obtained from multiple sources: National Tissue Resource Centre, Sun Health Research Institute, National Health and Medical Research Council Brain Bank and the Department of Pathology, University of Sydney, as previously described (Section 2.1).

3.2.2 Immunohistochemistry and analysis of apoptotic-like nuclei

Fourteen AD cases (average age, age range; average postmortem interval, postmortem interval range 76.8, 65-92 years; 10.7, 2-61 hours; Table 3.1), six preclinical AD cases (82.5, 71-91 years; 7.7; 2.25-32.5 hours; Table 3.1) and four control cases (55.3, 47-65 years; 23.5, 16-27.5 hours; Table 3.1) were stained with thioflavine s (Section 2.2.1) and Nuclear Yellow (Section 2.2.2). Cases were age and PMI matched as closely as possible, however, the age and PMI of control cases was significantly different when compared to AD cases, and the age of the control cases was also significantly different lower than preclinical cases ($p < 0.05$). It was not possible to gender match between case types due to the difficulty in obtaining appropriately fixed human brain tissue. Although there was no robust association between sex and the percentage of apoptotic nuclei, an interaction between these two factors cannot be discounted nor could it be statistically analysed with this sample of cases. Thioflavine s stains plaques that contain fibrillar aggregates. Additionally, the SFG neocortex of five AD, five preclinical AD and four control cases and the ITG of five AD cases were quenched (as per Section 2.3.2), stained with thioflavine s (Section 2.2.1) and Nuclear Yellow (Section 2.2.2) and then immunolabelling for astrocytes (anti-glial fibrillary acidic protein, GFAP), oligodendrocytes (anti-oligodendrocyte/myelin specific protein), microglia (anti-ferritin), calretinin-labelled neurons or dephosphorylated NF triplet protein-labelled neurons (a pyramidal cell marker, see Kirkcaldie et al., 2002) and tau (Table 2.2) was performed as previously described (Section 2.3.3). Several AD brain tissue sections were also treated with 90% formic acid (Section 2.3.1) and labelled with rabbit anti-Aβ (Table 2.2) instead of thioflavine s-staining to more clearly assess the association of nuclei with Aβ plaques.

Table 3.1 Human cases used for cell type specific apoptotic-like nuclei analysis

Type	Age (years)	Gender	Postmortem interval (h)	Cortical region	Pathological diagnosis
AD	65	M	3	SFG, ITG	AD
AD	67	M	61	ITG	AD
AD	71	F	13	SFG, ITG	AD
AD	72	F	4	SFG, ITG	AD
AD	73	M	6.5	SFG, ITG	AD
AD	73	M	35	ITG	Pneumonia
AD	74	F	2	SFG, ITG	Pneumonia
AD	74	M	2.75	SFG	Respiratory failure, AD
AD	76	F	2.6	SFG, ITG	AD
AD	83	M	2.83	SFG, ITG	Dementia
AD	83	F	5	ITG	AD
AD	84	F	3	SFG, ITG	AD
AD	88	M	7	SFG, ITG	Dementia
AD	92	F	2.25	ITG	Pneumonia
Preclinical AD	71	M	32.5	SFG	Cardiac arrhythmia
Preclinical AD	78	M	2.25	SFG	Postoperative
Preclinical AD	81	F	3	SFG	Cardiac arrest
Preclinical AD	84	M	3	SFG	Cardiopulmonary arrest

Type	Age (years)	Gender	Postmortem interval (h)	Cortical region	Pathological diagnosis
Preclinical AD	90	M	2.16	SFG	Respiratory arrest
Preclinical AD	91	M	3	SFG	Cardiac failure
Control	51	M	23.3	SFG	Pulmonary embolus
Control	58	M	27	SFG	Coronary disease
Control	65	M	16	SFG	Cardiac infarction
Control	47	M	27.5	SFG	Cardiac infarction

To determine the percentage of abnormal nuclei present in pathology-rich cortical areas, images of five random fields of view in cortical layer III were captured at 40x in the SFG of six preclinical AD, six AD and four control cases and in the ITG of six AD, five preclinical AD and three control cases. Cases were selected based on optimal fixation conditions for Nuclear Yellow-staining. Normal and abnormal nuclei were counted, with apoptotic-like nuclei being defined as brightly fluorescent (indicating condensed chromatin), rounded, shrunken and/or fragmented nuclei (Tompkins et al., 1997; Verdageur et al., 2002; Conti et al., 2003; Roy and Sapolsky, 2003). To provide an indication of the severity of AD pathology, NFTs were counted in 10 randomly chosen fields of view in neocortical layer III of each case. Linear regression analysis was performed on the apoptotic nuclei, NFT load and postmortem interval data sets and no significant correlations were present. The nuclei of all tau-immunoreactive intracellular NFTs co-localised with dephosphorylated NF triplet protein-labelled neurons were also examined in the SFG and ITG of five AD cases.

Additionally, blinded to case type, 100 astrocytes, microglia, calretinin- and dephosphorylated NF triplet protein-labelled neurons were counted in each case and any co-localisation with apoptotic nuclei was recorded. As oligodendrocyte-specific-protein-labelling was often very dense in the outer neocortical layers quantitation of this cell type was not possible.

3.2.3 Immunohistochemistry and analysis of apoptosis-related proteins

To ascertain whether apoptotic pathways were activated in AD, sections from five AD (average age, age range, average postmortem interval, postmortem interval range; 76, 60-91 years; 41, 23.5-64.5 hours), five preclinical AD (81, 74-91 years; 49, 31.5-68 hours) and five control cases (74, 58-84 years; 44, 26.5-57 hours) underwent antigen retrieval (Section 2.3.4) and then immunohistochemistry for aC3, aC8, aC9, Bcl-2, Bax or TRADD (Table 2.2) using standard immunoperoxidase labelling techniques (Section 2.3.5). The ages and PMIs of control, preclinical AD and AD cases were not significantly different. It was not possible to gender match between case types due to

the difficulty in obtaining human brain tissue. Although there was no robust association between sex and the percentage of the levels of apoptotic-related proteins and real time RT-PCR analysis, an interaction between these two factors cannot be discounted. However, such an interaction could not be statistically analysed with the sample of cases used in the current study. To determine the identity of the aC3-labelled cells the SFG neocortex of five AD, five preclinical AD and four control cases and the ITG of five AD cases were also double labelled with aC3 and GFAP, using standard immunofluorescent labelling techniques (Section 2.3.2) and stained with Nuclear Yellow (Section 2.2.2).

Whilst blinded to case type, each case was given a rating of 1-3 according to the amount of aC3-, aC8-, aC9-, Bcl-2-, Bax- or TRADD-labelling that was present in the neocortex in a similar way to previous studies (Lucassen et al., 1997; Stadelmann et al., 1998; Su et al., 2003). The tissue sections were then grouped into control, preclinical AD and AD cases and analysed by a one-way ANOVA with a Bonferroni post-hoc analysis. Following this, the brain sections were analysed for any change in the distribution of labelling for each apoptotic marker.

3.2.4 RNA extraction and purification

RNA was extracted from the frozen unfixed ITG neocortex of the same five AD (average age, age range, average postmortem interval, postmortem interval range; 76, 60-91 years; 41, 23.5-64.5 hours), five preclinical AD (81, 74-91 years; 49, 31.5-68 hours) and five control cases (74, 58-84 years; 44, 26.5-57 hours) that were immunolabelled for apoptotic related-proteins (Section 3.1.3). A thin slice of neocortex (0.04-0.1g) was taken from each case and the white matter and meninges were removed. RNA was extracted from the tissue samples using TRIzol® reagent (Invitrogen, Calsbad, CA) and then DNase treated with the DNA-free™ Kit (Ambicon, Austin, Texis) according to manufacturers instructions and stored at -80°C. The concentration and purity of the RNA solutions were determined by reading the optical density at 260 and 280nm of each sample diluted in TE buffer (pH 8.0). When RNA was extracted twice from the same cerebellum sample and analysed by RT-PCR less than five percent variation was present in the C_T value.

3.2.5 Oligonucleotide primers

The primer sequences used were as follows: Bax forward 5'-ATCCAGGATCGAGCAGGGCG-3' and reverse 5'-ACTCGCTCAGCTTCTTGGTG-3' (Billbault et al., 2004); Bcl-2 forward 5'-TGTGGCCTTCTTTGAGTTCG-3' and reverse 5'-GAAATCAAACAGAGGCCGCATG-3' (Sawa et al., 1997), TRADD forward 5'-CGGCTCCGGGATGAAGA-3'and reverse 5'-GAGCCGCACTTCAGATTTCG- 3' (Lund et al., 2003); GAPDH forward 5'-TTCATTGACCTCAACTAC-3'and reverse 5'-GTGGCAGTGATGGCATGGAC-3' (designed on Primer Express Software; Applied Biosystems Inc. Foster City, CA)

3.2.6 Real time RT-PCR

Real time quantitative RT-PCR reactions were carried out using the Quantitect® SYBR® Green RT-PCR kit (Qiagen; Applied Biosystems Inc. Foster City, CA) in a 10µl volume on a Rotor-gene 2000 (Corbett Research, Mortlake, NSW, Australia). All reactions were performed in duplicate with a no template control. The RT-PCR conditions for GAPDH and TRADD were 50°C for 30 min, 95°C for 15 min, followed by 40 cycles of 94°C for 15s, 55°C for 20s and 72°C for 20s. Whereas, the RT-PCR conditions for Bcl-2 and Bax were 50°C for 30 min, 95°C for 15 min followed by 40 cycles of 94°C for 15s, 50°C for 20s and 72°C for 20s. The fluorescence was recorded during the elongation phase of each PCR cycle. A melt curve from 65-95°C was also performed at the end of each RT-PCR run to ensure that no primer dimers were present in the reaction products.

A standard curve was prepared using real time RT-PCR products derived from cDNA for each primer set to ensure that the reaction was efficient over the range of concentrations present in the RNA samples. Briefly, real time RT-PCR products were run on a 2.5% agarose gel with a 100bp DNA Ladder (New England Biolabs, Herts, UK). The bands were photographed and excised under a UV transilluminator and cDNA was extracted with a QIAquick® gel extraction kit (Qiagen, Clifford Hill, Victoria, Australia) according to the manufacturer's protocol. A serial dilution of the

extracted cDNA was prepared for each primer set to create a RT-PCR cDNA standard curve. A standard curve was also prepared for each primer set using serial dilutions of brain RNA to ensure that the amount of RNA added to each RT-PCR reaction was not inhibiting the efficiency of the RT-PCR reaction.

The CT values of all samples for the Bax, Bcl-2 and TRADD primer sets were normalised to GAPDH by dividing the C_T value of the gene of interest by the C_T value of GAPDH for each sample. GAPDH was used as the housekeeping gene as it was similarly expressed in control and AD brains (Gutala and Reddy, 2004). The GAPDH normalised C_T values for each gene of interest in preclinical AD- and AD-samples were then divided by the average C_T value of the control samples and expressed as a percentage of the average control value. The data was analysed using a one-way ANOVA with a Bonferroni post-hoc analysis. When linear regression analysis was performed no significant correlation was present between postmortem interval and Bax, Bcl-2 and TRADD mRNA levels. In addition, no real time RT-PCR for caspases-3, -8 and -9 was performed as they are constitutively present as zymogens within cells, and as there was no qualitative correlation with amount of active caspase-3, -8 and -9 immunolabelling with case types, the unfixed frozen human brain tissue, which was a highly limited resource, was not utilised to determine caspase activity levels.

3.3 RESULTS

3.3.1 Apoptotic-like nuclei and cell type in AD, preclinical AD and control brains

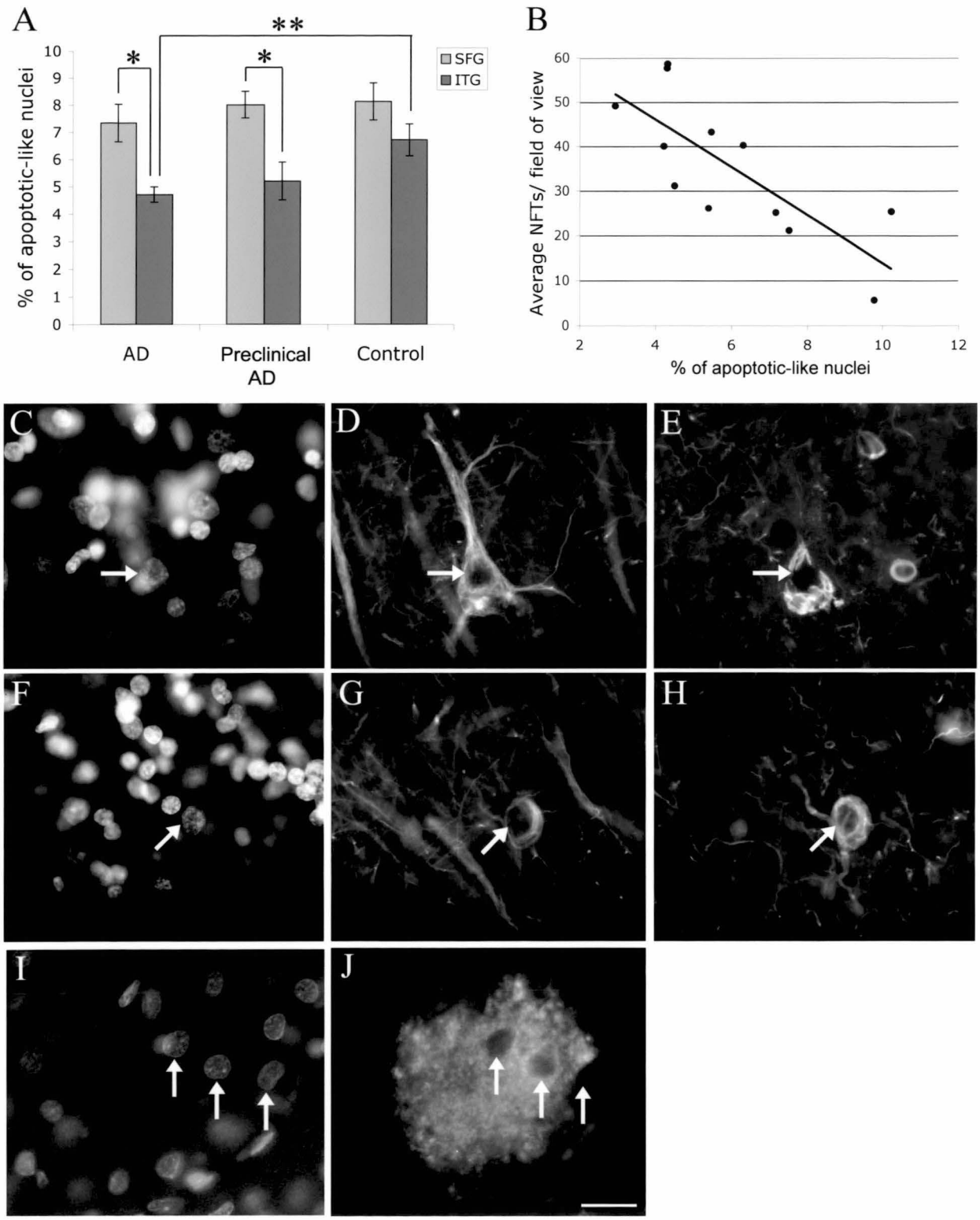
To assess the rate of apoptosis in AD, preclinical AD and control brains the percentage of apoptotic-like nuclei in neocortical layer III was investigated (Figure 3.1A). Statistical analysis demonstrated similar levels of apoptotic-like nuclei in neocortical layer III of the ITG in control and preclinical AD brains with significantly more abnormal nuclei in control cases than AD cases ($p = < 0.05$; Figure 3.1A). Additionally, the AD and preclinical AD cases had a significantly higher percentage of abnormal nuclei in cortical layer III in the SFG than in the ITG ($p = < 0.05$). In layer III of the ITG the percentages of cells with apoptotic-like nuclei in AD, preclinical AD and control cases were 4.72% ± 0.28, 5.20% ± 0.69 and 6.72% ± 0.59, respectively (Figure 3.1A). In layer III of the SFG 7.35% ± 0.69, 8.02% ± 0.49 and 8.13% ± 0.69 of cells were apoptotic-like in AD, preclinical AD and control cases, respectively (Figure 3.1A). The percentages of apoptotic nuclei in neocortical areas did not correlate with the postmortem intervals or age of the analysed cases ($p > 0.05$), and was not associated with particular modes or causes of death.

With specific reference to the hallmarks of AD, there was a significant negative correlation between the average number of NFTs in neocortical layer III and the percentage of apoptotic-like nuclei present ($p = < 0.05$, Figure 3.1B). In addition none of the dephosphorylated NF triplet protein-labelled NFT-bearing neurons (n=142) observed in the SFG and ITG of five AD cases had apoptotic-like nuclei, indicating that NFT-bearing neurons do not account for a high proportion of cells with apoptotic-like nuclei (Figure 3.1C-H). Nuclei observed adjacent to Aβ plaques and nuclei enveloped within Aβ plaque's fibrils were almost always non-apoptotic (Figure 3.1I-J).

To determine what type of cell exhibited apoptotic-like nuclei, GFAP, ferritin, calretinin, dephosphorylated NF triplet protein, and oligodendrocyte/myelin specific protein-

Figure 3.1

The percentage of apoptotic-like nuclei were not significantly different in neocortical layer III of the SFG in AD, preclinical AD and control cases or in the ITG of control and preclinical AD cases. Bar graph showing the percentage of apoptotic-like nuclei in neocortical layer III of the ITG and SFG of control, preclinical AD and AD cases (**A**: * $p < 0.001$ ** $p < 0.01$; error bars indicate SEM). Line graph demonstrating the significant negative correlation that was present when the average number of NFTs present per field of view in neocortical layer III of the SFG and ITG of each AD case were plotted against the average percentage of apoptotic-like nuclei present in neocortical layer III of each AD case (**B** $p = <0.001$; $R^2 = 0.7058$). Panels C-H show typical NFT-bearing neurons. None of the Nuclear Yellow-stained nuclei (arrows, **C, F**) of dephosphorylated NF triplet protein-labelled neurons (**D, G**) bearing tau-labelled intracellular NFTs (**E, H**) were apoptotic-like. I and J show Nuclear Yellow-stained nuclei (arrows, **I**) relative to a Aβ-labelled plaque (**J**), such nuclei almost always had normal morphology. Scale bar: C-H = 20μm; I-J = 10μm.


A
**
*
*
SFG
ITG
% of apoptotic-like nuclei
0
1
2
3
4
5
6
7
8
9
10
AD
Preclinical AD
Control
B
Average NFTs/ field of view
0
10
20
30
40
50
60
2
4
6
8
10
12
% of apoptotic-like nuclei
C
D
E
F
G
H
I
J

labelling was undertaken in conjunction with Nuclear Yellow-staining to identify astrocytes, microglia, calretinin-labelled neurons, dephosphorylated NF triplet protein-labelled neurons and oligodendrocytes, respectively (Figure 3.2). Frank nuclear apoptotic changes were largely restricted to astrocytes and microglia rather than calretinin- and dephosphorylated NF triplet protein-labelled neurons in AD, preclinical AD and control cases (Table 3.2). Apoptotic-like nuclei were consistently associated with abnormal cellular morphology (Figure 3.2).

3.3.2 Active-caspase-3-, active caspase-8-, active caspase–9-, Bax-, Bcl-2- and TRADD-labelling in AD, preclinical AD and control brains

The labelling of several apoptotic markers across AD, preclinical AD and control cases was also investigated. No increased labelling of any marker or different cortical or cellular labelling pattern that distinguished between case categories was present. When analysed blind to case type, there was no difference in the frequency of TRADD-, pro-apoptotic aC3-, aC8-, aC9- and Bax- and anti-apoptotic Bcl-2-labelling between control, preclinical AD or AD cases (Table 3.3). There was also no association between increased labelling of these apoptotic markers and the postmortem interval of each case (Table 3.3). Bax, Bcl-2, aC8, aC9 and TRADD immunohistochemical labelling were present in the soma of both pyramidal and non-pyramidal cells and were cytoplasmic apart from the granular labelling of TRADD (Figure 3.3). Bax, Bcl-2 aC8, aC9 and TRADD immunostaining was present in the proximal region of the apical dendrites of some pyramidal cells and aC9-, Bax- and TRADD-labelling also extended into the basal dendrites of some pyramidal cells (Figure 3.3). Active caspase-3-labelling was also cytoplasmic, but in contrast to the other apoptotic-related proteins, aC3-labelled the soma of glial cells (Figure 3.4). Active caspase-3-labelling co-localised with GFAP (Figure 3.4). Additionally, co-staining with Nuclear Yellow demonstrated that the nuclei of > 95% of aC3-labelled cells were not apoptotic-like.

Active caspase-9-labelling was observed throughout neocortical layers II-VI with an increased number of labelled cells in layers III and V in all cases. Similarly, Bcl-2

Figure 3.2

There was no significant difference in the percentage of astrocytes, microglia and neuronal subsets with apoptotic-like nuclei in the neocortex of control, preclinical AD and AD cases. Apoptotic-like nuclei were defined as brightly fluorescent, rounded, shrunken (arrow, **C**) and/or fragmented (arrow, **D**) nuclei. All of the dephosphorylated NF triplet protein-labelled neurons (**A**) and most of the ferritin-labelled microglia (**E**), GFAP-labelled astrocytes (**I**), oligodendrocytes (**M**) and calretinin-labelled neurons (**Q**) had normal nuclei (arrows, **B, F, J, N, R**, respectively) and cellular morphology (**A, E, I, M, Q**, respectively) in the neocortex. The small percentage of microglia, astrocytes, oligodendrocytes and calretinin-labelled neurons with apoptotic-like nuclei (arrows, **H, L, P, T**, respectively) also exhibited abnormal cellular morphology (**G, K, O, S**, respectively) characterised by cytoplasmic shrinkage. Scale bar: A-B = 5μm; C-T = 10μm.

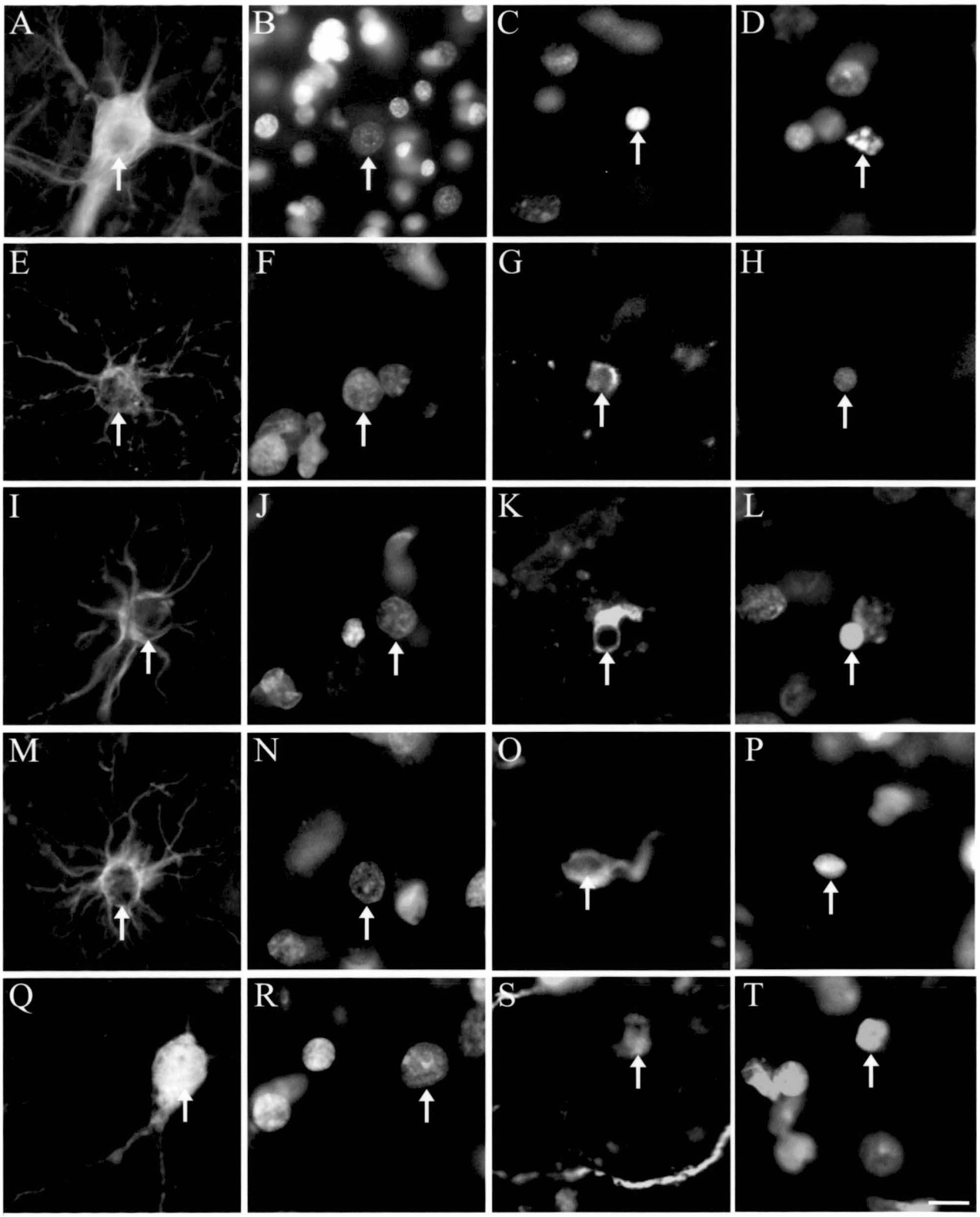

A
B
C
D
E
F
G
H
I
J
K
L
M
N
O
P
Q
R
S
T

Figure 3.3

No robust changes in the labelling of apoptotic-markers were present in the neocortex of control, preclinical AD and AD cases. Labelling for TRADD (**A**), aC8 (**B**), aC9 (**C**), Bax (**D**) and Bcl-2 (**E**) in neocortical layer III of all case types was somatic and present in both pyramidal and non-pyramidal cells. Labelling of all the apoptotic markers often extended into the proximal apical dendrites (arrows) of pyramidal cells. While TRADD-, aC9- and Bax-labelling was also observed to extend into the basal dendrites (arrow heads) of pyramidal cells. The laminar distribution of TRADD (**F**), aC9, Bax and Bcl-2 were similar in most of the cases examined, and exhibited an increased density of labelling in neocortical layers III and V. Scale bar: A-E = 45μm; F = 100μm.

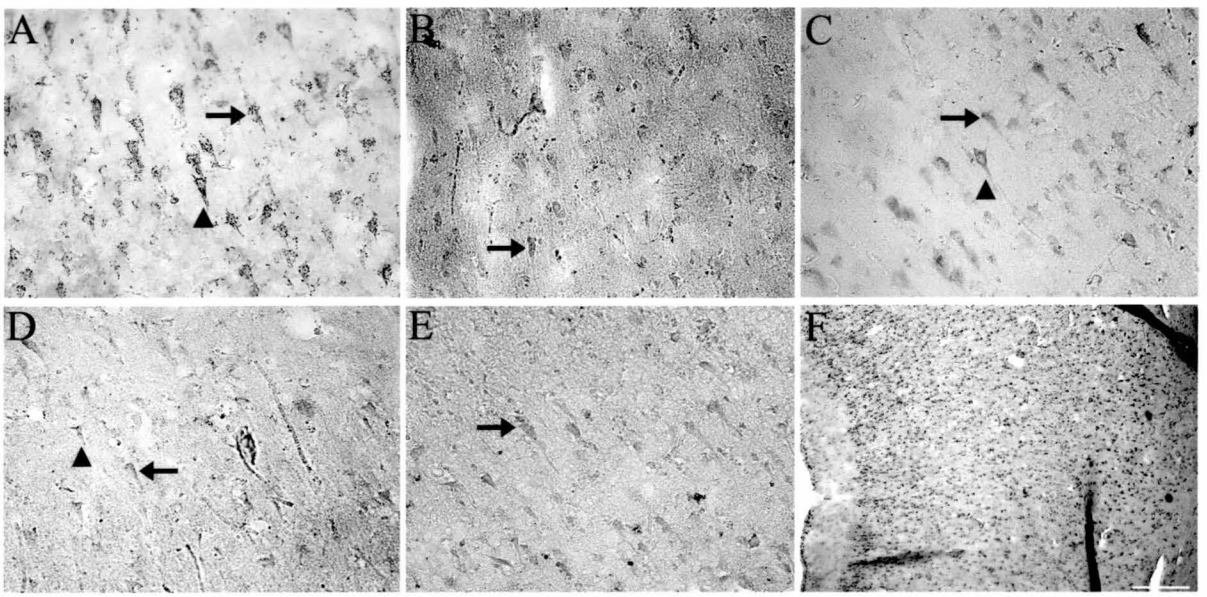

A
B
C
D
E
F

Figure 3.4

Labelling for aC3 in neocortical layer III of all case types was cytoplasmic (**A**) and present in glial cells (arrows). Active caspase-3-labelling (**B**) co-localised with GFAP (**C**) and extended into the processes (arrows) of most aC3-labelled cells. The laminar distribution of aC3-labelling was homologous (**D**) throughout neocortical layers I-VI in most cases. WM = white matter Scale bar: A = 170μm; B-C = 25μm; D = 330μm.

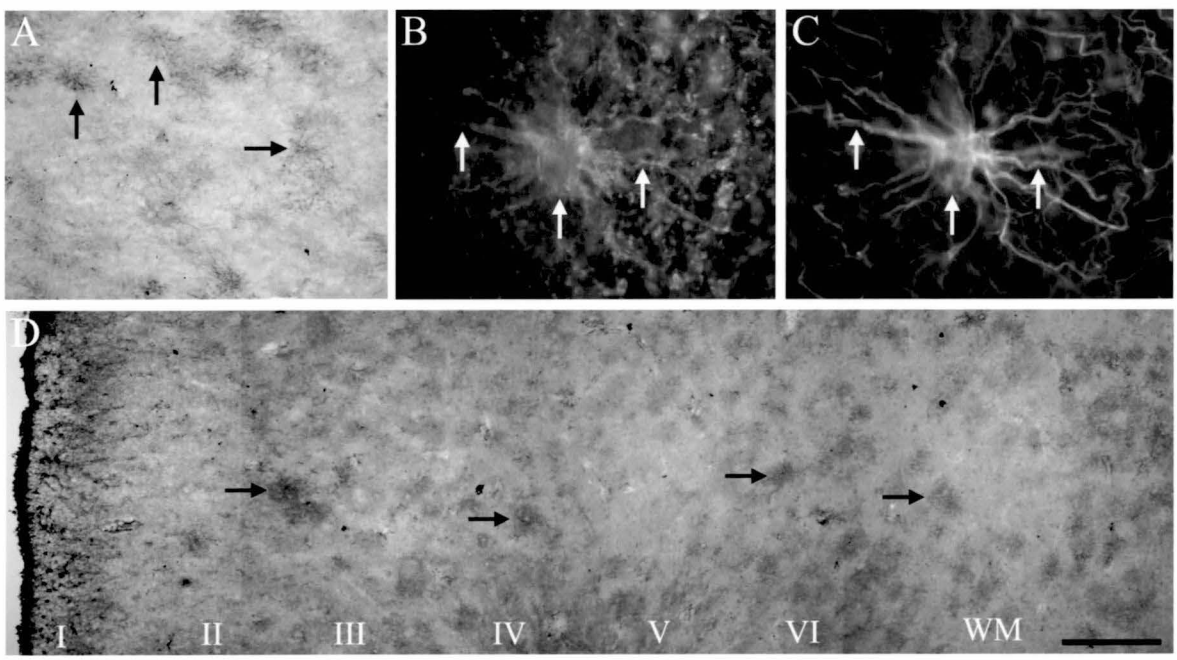

A
B
C
D
I
II
III
IV
V
VI
WM

Table 3.2 The percentage of astrocytes, microglia and neurons labelled with calretinin or dephosphorylated NF triplet proteins with apoptotic like-nuclei in layer III of the neocortex.

	Astrocytes (GFAP-labelled)	Microglia (ferritin-labelled)	Neurons (calretinin-labelled)	Neurons (dephosphorylated NF triplet protein-labelled)
AD SFG	0.6 ± 0.4	0.8 ± 0.6	0.0 ± 0.0	0.0 ± 0.0
Preclinical AD SFG	1.2 ± 0.7	0.2 ± 0.2	0.0 ± 0.0	0.0 ± 0.0
Control SFG	0.75 ± 0.5	0.5 ± 0.3	0.3 ± 0.2	0.0 ± 0.0
AD ITG	1.0 ± 0.3	1.4 ± 0.6	0.0 ± 0.0	0.0 ± 0.0

Table 3.3 Human brain cases used for analysis of apoptotic markers and RNA extraction.

Type	Age (years)	Gender	PMI (h)	Pathological Diagnosis	Immunohistochemical staining for: Active caspase-8	Active caspase-9	Active caspase-3	Bax	Bcl-2	TRADD
AD	60	M	64 5	COAD	+	+	++	+	++	+++
AD	67	M	31	Respiratory failure	+++	++	+++	+	+	+
AD	79	M	24	Respiratory failure	++	+++	+++	+++	+	+
AD	81	M	23 5	Cardiac failure	+++	++	+	+++	++	+++
AD	91	F	64	Malignant mesothelioma	+	+++	+++	+	+++	+
Preclinical AD	74	M	31 5	Cardiac failure	+	++	++	+	+++	++
Preclinical AD	74	M	68	Cardiac failure	++	+	++	++	++	++
Preclinical AD	82	M	48 5	Cardiac infarction	+++	+++	+	+	+	+++
Preclinical AD	82	M	50	Cardiac failure	+	+++	+++	++	+	+++
Preclinical AD	91	M	48	Renal failure	++	++	+	+++	+	+
Control	58	F	30	Asthma	+++	++	++	++	+	++
Control	73	F	26 5	Pulmonary Embolism	+++	+	++	+	++	+++
Control	77	M	53 5	Cardiac failure	+++	++	+	+++	+++	+++
Control	79	M	57	Respiratory failure	+	+	+++	++	+++	++
Control	84	M	55	Cardiac infarction	++	+++	+	++	++	+

PMI, postmortem interval

labelling was present in neocortical layers I-VI and the density of labelled cells was increased in layers III and V in most cases. Bax immunoreactivity was distributed throughout neocortical layers II-VI with an increased density of labelled cells in neocortical layer III in most cases. TRADD immunoreactivity was also observed in neocortical layers II-VI and an increase in the number of TRADD-labelled cells was present in layers III and V in some cases (Figure 3.3F). In contrast, aC8-labelling was homologous throughout neocortical layers III-VI in all cases, while aC3-labelling was homologous throughout layers I-VI in most cases. The density of Bcl-2, aC9 and TRADD-labelling in neocortical layers III and V was variable, being higher in layer III than in layer V in some cases and higher in layer V than in layer III in others.

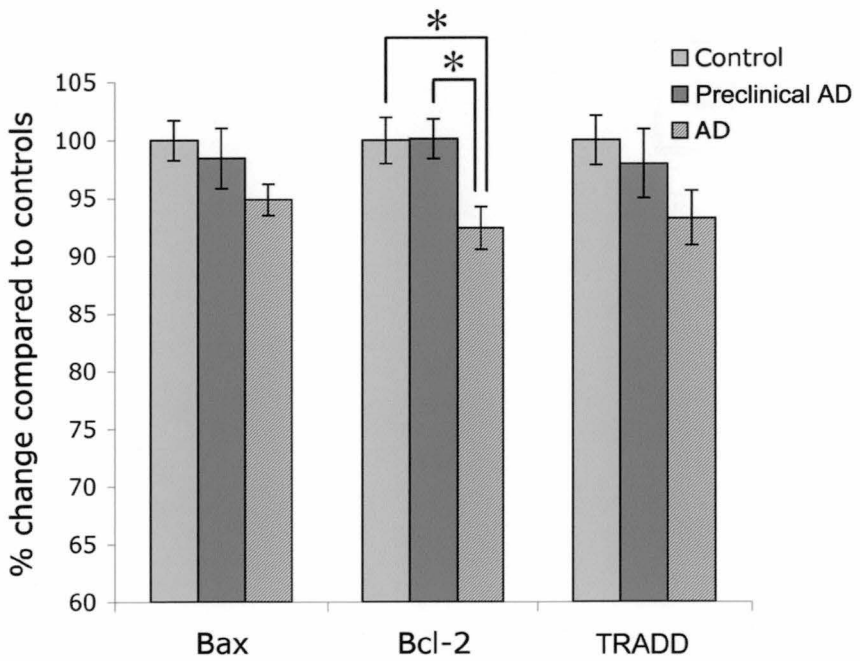
3.3.3 Real time RT-PCR analysis of Bax, Bcl-2 and TRADD in AD, preclinical AD, and control cases

To confirm the immunohistochemical observations of apoptotic markers in the preclinical AD, AD and control brains, the mRNA levels of Bax, Bcl-2 and TRADD were analysed in the same set of cases. No dramatic changes in the expression of these three genes were found, although there was a trend of reduced expression of each gene (relative to GAPDH) in AD brains compared to control brains that attained statistical significance in the case of Bcl-2 (Figure 3.5). No difference in Bcl-2 mRNA levels were present between control and preclinical AD cases, but there was a small but significant decrease in Bcl-2 mRNA in AD cases to 92.4% of control levels when control and preclinical AD cases were compared to AD cases (Figure 3.5, $p = < 0.05$).

When analysed by real time RT-PCR, the mRNA levels of Bax were decreased to 98.4 ± 2.6% and 94.9 ± 1.3% of control Bax levels in preclinical AD and AD cases, respectively (Figure 3.5). The levels of Bcl-2 mRNA was decreased to 92.4 ± 1.8% of control levels in AD cases, while the level of Bcl-2 mRNA in preclinical AD cases was increased to 100.1 ± 1.7% of control Bcl-2 levels. TRADD mRNA levels were decreased to 98.0 ± 3.0% and 93.3 ± 2.3% of control TRADD levels in preclinical AD and AD cases, respectively (Figure 3.5).

Figure 3.5

There was little difference between Bax, Blc-2 and TRADD mRNA levels in control, preclinical AD and AD cases. Bar graph showing the percentage change of Bax, Bcl-2 and TRADD mRNA extracted from the ITG neocortex in preclinical AD and AD cases compared to the control cases. * $p<0.05$. Error bars indicate SEM.


*
*
Control
Preclinical AD
AD
% change compared to controls
105
100
95
90
85
80
75
70
65
60
Bax
Bcl-2
TRADD

3.4 DISCUSSION

Using a combination of immunohistochemical and molecular techniques, the degree of apoptosis in AD, preclinical AD, and control brains was investigated. Overall there were no robust changes in the presence of apoptotic-related proteins or in the percentage of apoptotic nuclei between control and AD cases. Analysis of the preclinical cohort of cases also showed no obvious increases or decreases in apoptotic markers or apoptotic-like nuclei. These results suggest that there is no staging of apoptotic changes in AD and that apoptosis does not play a major role in AD pathogenesis, although apoptosis may still be involved in AD-associated neurodegeneration.

Unlike previous studies (Satou et al., 1995; MacGibbon et al., 1997; Nagy and Esiri, 1997; Su et al., 1997; Kitamura et al., 1998; Giannakopolous et al., 1999; Rohn et al., 2001a; Rohn et al., 2002; Zhao et al., 2003b; Del Villar and Miller, 2004), the current investigation assessed a wide range of apoptotic-markers including aC3, aC8, aC9, Bax, Bcl-2 and TRADD in the same set of control, AD and preclinical AD cases and observed no consistent alterations in the frequency, or cortical and cellular localisation of labelling between case types. Although, a few research groups have reported similar levels of Bax (Kitamura et al., 1998) and Bcl-2 (Nagy and Esiri, 1997; Stadelmann et al., 1998) in AD and control brains. Additionally, the unique ability to analyse the same cohort of control, preclinical AD and AD cases by real time RT-PCR provided data that supported the immunohistochemical results. No major change in the expression of Bax, Bcl-2 and TRADD mRNA levels were detected apart from significant small decrease of Bcl-2 mRNA to 92.4% of control levels. The importance of the small decrease in Bcl-2 mRNA is difficult to assess as these results represent a contribution from all expressing cells in the neocortex and are influenced by the progressive neuronal loss and increasing numbers of reactive astrocytes and activated microglia in the cortex in AD (Schechter et al., 1981; Hof et al., 1990; Szpak et al., 2001).

Abnormal nuclei were distributed amongst glial cell types and the calretinin-labelled subset of neurons (Sampson et al., 1997), and labelling for aC8, aC9, Bax, Bcl-2 and TRADD were also present in glia and neurons in all case types. However, there were no

significant differences in apoptotic marker labelling or the rate of apoptosis of astrocytes, microglia and calretinin- and dephosphorylated NF triplet protein-labelled neurons between AD, preclinical AD and control cases. These results elaborate on previous reports of DNA fragmentation (Smale et al., 1995; Troncoso et al., 1996; Lucassen et al., 1997; Sugaya et al., 1997; Masliah et al., 1998; Jellinger and Stadelmann, 2001; Kobayashi et al., 2002; Kobayashi et al., 2004) and apoptotic-related proteins (MacGibbon et al., 1997; Nagy and Esiri, 1997; Su et al., 1997; Kitamura et al., 1998; Masliah et al., 1998; Stadelmann et al., 1999; Su et al., 2002; Zhao et al., 2003a; Zhao et al., 2003b) localised in glia and neurons in control and AD cases. The cellular and cortical localisation and clear labelling of pyramidal cells in neocortical layers III and V observed for Bax, Bcl-2, aC8, aC9 and TRADD was similar to reports in previous studies (MacGibbon et al., 1997; Su et al., 1997; Masliah et al., 1998; Rohn et al., 2001a; Rohn et al., 2002; Su et al., 2002; Zhao et al., 2003a), and may indicate that the pyramidal subset of neurons are especially vulnerable to molecular and mechanical insults. However, despite pyramidal neurons being selectively vulnerable to degeneration in AD (Hof et al., 1990; Hof, 1997) and clearly immunolabelled for apoptotic-related proteins, both dephosphorylated NF triplet protein-labelled pyramidal cells and calretinin-labelled interneurons rarely co-localised with apoptotic-like nuclei in any case type. Stadelmann et al. (1999) have previously documented aC3-labelling in approximately 1 in 1100 to 5000 neurons in the hippocampal formation of AD cases but not controls cases. Similarly, using the same antibody as the present study, Gastard et al. (2003) described aC3-labelling in the entorhinal cortex and the hippocampus. In contrast, the current study determined that aC3-labelling in two neocortical areas was associated with glial cells and not neurons, with glial cells similarly demonstrating a higher percentage of apoptotic-like nuclei than the calretinin- and dephosphorylated NF triplet protein-labelled neurons in all case types. Indeed, no pyramidal or non-pyramidal neurons were detected in the neocortex labelled with these markers with frank nuclear changes indicative of apoptosis in any AD or preclinical AD cases, indicating a frequency of apoptosis much less in the neocortex than in the hippocampal formation (Stadelmann et al., 1999). This may reflect a differential staging of the disease between

these brain regions, with the hippocampal formation and entorhinal cortex demonstrating substantial pathology much earlier than the neocortex (reviewed in Vickers et al. 2000).

The low percentage of apoptotic nuclei present in neocortical layer III of the ITG and SFG in control, preclinical AD and AD cases confirms previous qualitative reports of classic apoptotic morphology being seldom observed in AD brains (Lassmann et al., 1995; Troncoso et al., 1996; Lucassen et al., 1997; Stadelmann et al., 1998; Jellinger and Stadelmann, 2000; Nunomura and Chiba, 2000; Raina et al., 2003). The percentage of apoptotic nuclei in each case demonstrated no relationship with postmortem intervals. Unexpectedly, the percentage of apoptotic-like nuclei was significantly increased by 2.0% in control cases compared to AD cases in the ITG. Statistical analysis also showed that the SFG of AD and preclinical AD cases had a significantly higher percentage of abnormal nuclei in cortical layer III than the ITG. Additionally, there was also an inverse correlation between NFT quantity and apoptotic nuclei. This result was unanticipated as the ITG typically has more NFT-bearing neurons than the SFG (Braak and Braak, 1991; Vickers et al., 2003). Finally, there was no clear association between the apoptotic profile of cases relative to the mode or cause of death. However, it cannot be ruled out definitively as to whether variance in premortem illness or agonal state may underlie the small differences between control brains and cases showing AD-related pathology. Cardiac and respiratory failures were relatively more common in the control cases, raising the possibility of brain ischemia contributing to these differences. Conversely, given that the frank apoptotic pathology is restricted to glial cell types, these data may indicate a lower degree of normal turnover of these cells in response to active neurodegeneration in AD cases.

The lack of any specific co-localisation of apoptotic-like nuclei with pathological hallmarks of AD such as NFTs or Aβ plaques is a striking finding (Troncoso et al., 1996; Lucassen et al., 1997; Sugaya et al., 1997; Broe et al., 2001). The paucity of apoptotic-like nuclei within or adjacent to Aβ plaques provides some *in vivo* evidence against the proposition that Aβ plaques are toxic to surrounding neurons. Surprisingly early- (Figure 3.1C-E) and late-stage (Figure 3.1F-H) NFTs were not co-localised with

apoptotic-like nuclei. In conjunction with reports that most neocortical NFTs in AD are intracellular (Sampson et al., 1997; Vickers et al., 2003) and recent evidence that NFTs in experimental models do not directly correlate with neuronal loss (Andorfer et al., 2005; SantaCruz et al., 2005), these findings suggest that affected neurons may be able to withstand NFT formation for long periods of time before frank degeneration occurs (as reviewed by Jellinger and Stadelmann, 2001). However, one apoptosis related protein, cyto c, has been observed in a subset of neurons that exhibit pre-NFTs and a subset of Aβ plaque-associated DNs in AD cases (Blanchard et al., 2003).

The variability, discrepancies and contradictions present in the literature, including the data generated in the current study, regarding apoptotic nuclear morphology, DNA fragmentation and the level of apoptotic markers in AD and healthy control brains may be the result of different cerebral areas and cell populations being analysed, the different molecular and immunohistochemical techniques and/or antibodies used and even differences between fixation methods or the exact cohort of brain cases analysed (eg. Table 1.3). Thus, this comprehensive analysis of several apoptotic-related proteins via immunohistochemistry and real time RT-PCR, apoptotic morphology and the association of apoptotic morphology with the pathological hallmarks of AD in a substantial sample of control, preclinical AD and AD cases has notably contributed to this contentious area of AD research.

In summary, there were no significant differences in the incidence of apoptotic nuclear morphology and apoptotic markers at the cellular and gene expression level, present in control, preclinical AD and AD brains. These data suggest apoptosis may not play a major role in the pathogenesis or widespread neuronal loss that occurs in AD. Furthermore, as the underlying cause of neuronal loss and the final pathway to cell death in AD are yet to be fully elucidated, expanding our knowledge of the cellular processes involved in AD progression that ultimately lead to the dysfunction and loss of nerve cells may be vital for the discovery or design of effective therapeutic agents for AD and other neurodegenerative diseases.

4 CYTOPLASMIC CYTOCHROME C IMMUNOLABELLING IN DYSTROPHIC NEURITES IN ALZHEIMER'S DISEASE

4.1 INTRODUCTION

Cytochrome c has a well-established role in electron transfer and as a mediator of apoptotic cell death. The release of cyto c occurs upon activation of the intrinsic apoptotic pathway, which is regulated by cytokines and other death-survival signals (as reviewed in Adams and Cory, 2001). Although cyto c release is indicative of the activation of the intrinsic apoptotic pathway, it may also indicate mitochondrial damage or dysfunction. Additionally, such cyto c release from mitochondria correlates with disease progression in transgenic mouse models of Huntington's disease and amyotrophic lateral sclerosis (Zhu et al., 2002; Wang et al., 2003b). Following release into the cytoplasm, cyto c is able to interact with Apaf-1, dATP and caspase-9 to form the apoptosome complex, which results in the activation of caspase-9 and the subsequent activation of effector caspases. In addition to cyto c playing a role in apoptosis, increased cyto c levels and the release of cyto c from mitochondria are also associated with mitochondrial damage and dysfunction, both of which may occur in association with oxidative stress in normal aging and AD progression (Gibson et al., 2000; Hirai et al., 2001; Pollack et al., 2002; Manczak et al., 2005; Sullivan and Brown, 2005; Manczak et al., 2006).

Thus, the correlation between cyto c-labelling and AD staging including the pathological hallmarks of AD has been further investigated, with particular reference to the intracellular localisation of cyto c. Using double-label fluorescence immunohistochemistry, the location of cyto c relative to the specific pathological hallmarks of AD, such as NFTs, DNs and Aβ plaques, were investigated in human control, preclinical AD and AD cases.

4.2 MATERIALS AND METHODS

4.2.1 Tissue source and processing

Human brain tissue was acquired from two sources: the Sun Health Research Institute and the University of Sydney, as previously described (Section 2.1). These samples included sections of the SFG from four control cases (average age, age range, average postmortem interval, postmortem interval range; 55.25, 47-65, 23.45, 16-27.5; Table 4.1), six preclinical AD cases (82.5, 71-91, 3-32.5, 7.65; Table 4.1) and six AD cases (82.5, 74-92, 3.3, 2.0-7.0; Table 4.1). The control cases exhibited significantly different ages and PMIs when compared to the preclnical AD and AD cases, largely due to the rarity of healthy aged brain material. It was also not possible to gender match between case types due to the difficulty in obtaining human brain tissue. Although there was no robust association between sex and the number of cytochrome c-labelled cells in the neocortex, an interaction between these two factors cannot be discounted nor could it be statistically analysed with this sample of cases.

4.2.2 Immunohistochemistry

The SFG of six AD cases, six preclinical AD cases and four control cases were double-immunolabelled for cyto c and human-tau, Aβ, NFM or chromogranin A (CgA) (Table 2.2) as previously described (Section 2.3.3). All cases were selected based on optimal fixation conditions for cyto c and NFM or CgA-labelling. Tissue sections were viewed on a Leica DMLB2 microscope with images captured on an Optronics Magnafire cooled CCD camera, as well as an Optiscan F900e krypton/argon confocal scanning system attached to an Olympus BX50 epifluorescence microscope.

4.2.3 Qualitative, quantitative and statistical analysis

Alzheimer's disease, preclinical AD and control tissue sections were analysed for any change in the cellular or neocortical distribution of cyto c-labelling. To determine the density of cyto c-labelled cells, images of 10 non-overlapping random fields of view in neocortical layer V were captured at 20x. For quantitation of the coexistence of

Table 4.1 Human cases used for cyto c double-labelling immunohistochemistry.

Type	Age (years)	Gender	Postmortem interval (h)	Pathological Diagnosis
AD	74	F	2	Pneumonia
AD	74	M	2.75	Respiratory failure, AD
AD	83	M	2.83	Dementia
AD	84	F	3	AD
AD	88	M	7	Dementia
AD	92	F	2.25	Pneumonia
Preclinical AD	71	M	32.5	Cardiac arrhythmia
Preclinical AD	78	M	2.25	Postoperative
Preclinical AD	81	F	3	Cardiac arrest
Preclinical AD	84	M	3	Cardiopulmonary arrest
Preclinical AD	90	M	2.16	Respiratory arrest
Preclinical AD	91	M	3	Cardiac failure
Control	47	M	27.5	Cardiac infarction
Control	51	M	23.3	Pulmonary embolus
Control	58	M	27	Coronary disease
Control	65	M	16	Cardiac infarction

cytoskeletal and synaptic elements and cyto c in DNs 100 Aβ plaques were analysed in the SFG of six AD cases and five preclinical AD cases. However, due to the presence of limited Aβ plaque numbers in some preclinical AD cases, 85 Aβ plaques were analysed for the co-localisation of CgA and cyto c in DNs across preclinical AD cases. Co-localisation of cyto c immunoreactivity and NFTs was also assessed by analysing 100 NFTs in the SFG of AD cases. The percentage of NFM, tau and CgA DNs, as well as NFTs, that co-localised with cyto c immunolabelling were calculated and all quantitative data was analysed by a two tailed t-test (two sample unequal variance) or a one-way ANOVA with a Bonferroni post-hoc analysis. Linear regression analysis was performed on the average number of cyto c-labelled cells, age and postmortem interval data and no significant correlations were present.

4.3 RESULTS

4.3.1 Cellular and neocortical distribution of cyto c

There were no changes in the cellular and neocortical localisation of cyto c between AD, preclinical AD and control cases examined in this study (Figure 4.1). In control, preclinical AD and AD cases cytochrome c-labelling was present in cells in neocortical layers II-VI, with a robust increase in the density of labelled cells in layers III and V (Figure 4.1A,B). In neocortical layers III and V, high levels of cyto c-labelling were present in a subset of pyramidal neurons in all case types (Figure 4.1). Clusters of cyto c-labelled pyramidal neurons (Figure 4.1C) were present in numerous cases across all case types, but were not specifically associated with Aβ plaques in preclinical AD and AD cases (Figure 4.1D). Cytochrome c immunolabelling was punctate and somatic, with labelling observed in the proximal portion of many cellular processes and extending into the distal regions of several processes in control, preclinical AD and AD cases (Figure 4.1E). Importantly, when cyto c-labelling was investigated utilising confocal microscopy across all case types, none of the cyto c-labelled cells observed exhibited non-punctate cytoplasmic staining, indicating that cyto c had not been released from mitochondria. The density of cyto c-labelled cells in neocortical layer V of the SFG did not differ significantly ($p > 0.05$) between case types (Figure 4.1F). The average number of cyto c-labelled cells did not correlate with the age or postmortem intervals ($p > 0.05$) or the particular modes or causes of death of the analysed cases.

4.3.2 Co-localisation of cyto c and AD-associated pathology

Analysis of cyto c immunoreactivity in relation to the pathological hallmarks of AD resulted in the detection of subsets of cyto c-labelled NFTs and DNs. The percentage of NFT-bearing neurons that were immunoreactive for cyto c in the neocortex of the SFG of AD cases was 6.7 ± 1.4% (Figure 4.2A). Moreover, cyto c-labelling was punctate in NFT-bearing neurons (Figure 4.2B,C) when assessed by scanning confocal microscopy. Similarly, a small percentage of tau-, NFM- and CgA-labelled DNs were also immunoreactive for cyto c in AD and preclinical AD cases (Figure 4.3, Table 4.2). In

Figure 4.1

The cortical and cellular distribution of cyto c-labelling was not substantially different between control, preclinical AD and AD cases. Cytochrome c-labelled cells were present at an increased density in layers III and V (**A**, layer III shown here). Cytochrome c-labelled cells were present at a lower density in other neocortical layers, such as layer II (**B**). Clusters of intensely labelled pyramidal neurons (arrows) in layer V of an AD case (**C**). Such clusters of cyto c-labelled neurons were evident in layers III and V of the neocortex in several cases. These clusters of intensely cyto c-labelled (green) pyramidal neurons (**D**, arrows) were not specifically associated with Aβ plaques (red, arrow heads). High magnification confocal microscopy confirmed that the cyto c-labelling present in neocortical cells was punctate (**E**). Bar graph demonstrating the average number of cytochrome-c-labelled cells per sample field of view in layer V across case types (**F**). There was no significant difference between the average number of cyto c-labelled cells in layer V of the SFG in control, AD or preclinical AD cases. Error bars indicate SEM. Scale bar: A, B, D = 40μm, C = 200μm, E = 14μm.

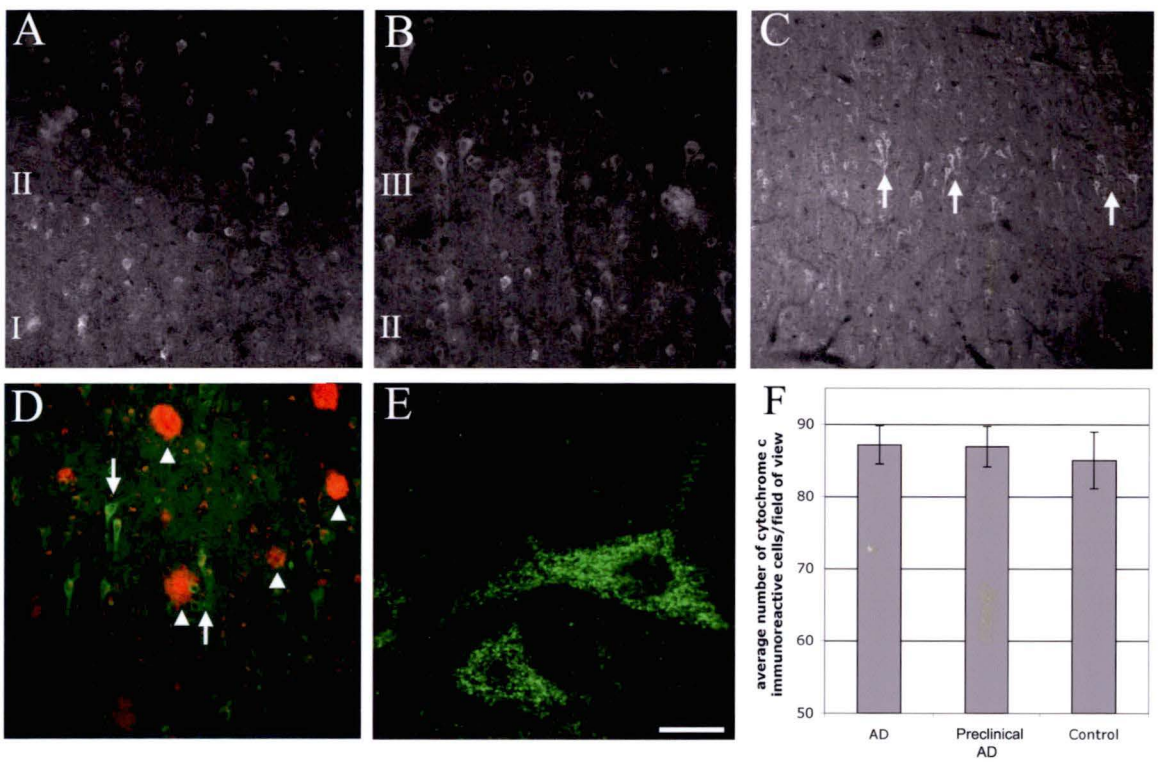

A
II
I
B
III
II
C
D
E
F
average number of cytochrome c immunoreactive cells/field of view
90
80
70
60
50
AD
Preclinical AD
Control

Figure 4.2

In AD cases cyto c immunolabelling was present in a subset of NFT-bearing neurons. A low percentage of tau-labelled NFTs (**A**, red, arrowheads) co-localised with cyto c-immunoreactivity (green, arrow). When observed with high magnification confocal microscopy NFT-bearing neurons (arrows) exhibited punctate cyto c-labelling (**B, C**). Scale bar: A = 10μm, B, C = 1μm.

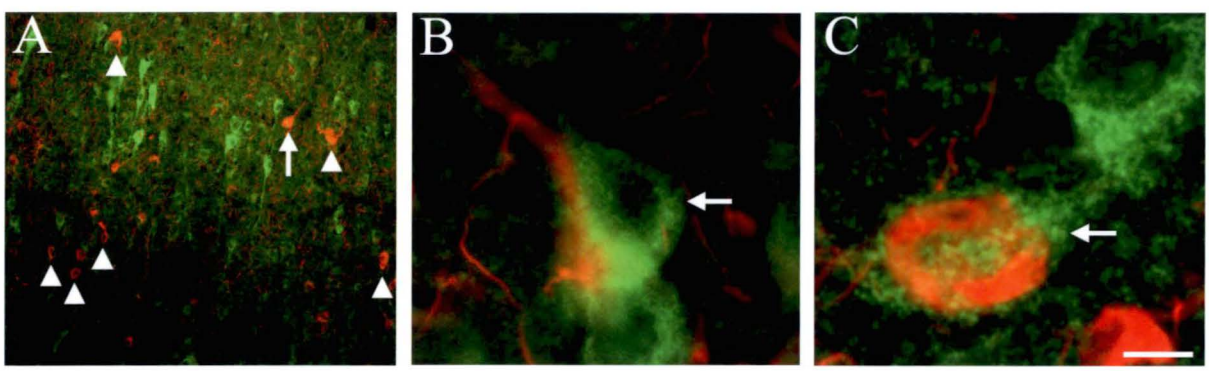
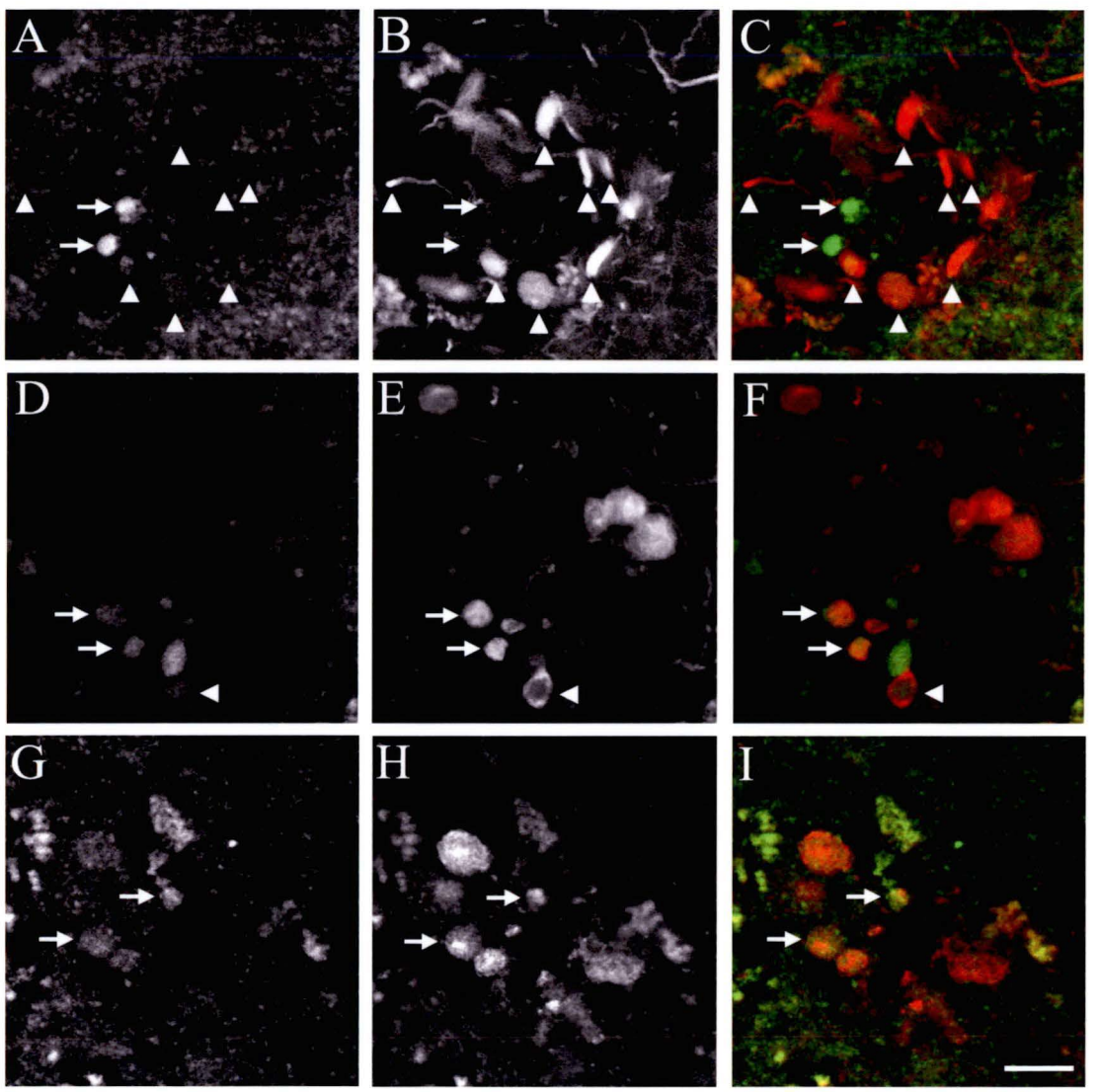

A
B
C

Figure 4.3

The cyto c-immunoreactivity present in DNs was often diffuse and co-localised with a subset of tau-, NFM- and CgA-labelled DNs. Cytochrome c-labelled DNs (**A**, arrows) were seldom co-localised with the tau-labelled DNs (**B,** arrow heads), when representative images were overlayed (**C**). Cytochrome c-labelling (**D**) was observed in a small percentage of NFM-labelled DNs (**E**, arrows), including some NFM-labelled DNs with a cyto c-immunoreactive central core (**F,** arrow head). Some cyto c-immunoreactivity (**G**) present within DNs (arrows) was punctate and also co-localised with a subset of CgA-labelled DNs (**H**, arrows), when representative images were overlayed (**I**). Scale bar = 11μm.


A
B
C
D
E
F
G
H
I

AD cases, an appreciably lower percentage of tau-labelled DNs co-localised with cyto c compared to NFM- and CgA-labelled DNs (Table 4.2). There was also a trend towards a lower percentage of NFM- and CgA-labelled DNs co-localising with cyto c in the SFG of AD cases compared to preclinical AD cases (Table 4.2). Tau-immunoreactive DNs were not present in the preclinical AD cases. Cytochrome c-labelled DNs were present in a subset of DN clusters and exhibited two generalised morphological variants. In AD and preclinical AD cases, bulbar cyto c-labelled DNs of variable sizes were observed most frequently, while elongated cyto c-labelled DNs were seldom observed (Figure 4.3). Additionally, NFM-labelled DNs with extensive cyto c-labelled central elements or caps of cyto c immunoreactivity were also present (Figure 4.3B). Strikingly, when cyto c-labelled DNs were examined using confocal scanning microscopy the labelling present was frequently cytoplasmic and not punctate (Figure 4.3).

Table 4.2 Co-localisation between cytoskeletal and synaptic markers and cyto c in Aβ plaque-associated DNs.

	mean % co-localisation ± SEM	
Labelling	Preclinical AD	AD
Tau with cyto c	-	1.44 ± 0.36
NFM with cyto c	3.63 ± 0.67	2.66 ± 0.76
CgA with cyto c	4.87 ± 0.55	2.55 ± 0.36

4.4 DISCUSSION

Cytochrome c-labelling was substantial in a subset of cortical neurons, with no difference in the cortical labelling pattern or the density of cyto c-labelled cells observed in control, preclinical AD and AD cases. Similar levels of cyto c have also been documented in the frontal cortex of AD and control cases using molecular methods (Engidawork et al., 2001), although one recent study reported increased cyto c immunolabelling within cortical neurons in AD compared to control cases (Blanchard et al., 2003). In this and previous studies, punctate cyto c immunoreactivity was observed in a subset of cortical cells (Blanchard et al., 2003; Manczak et al., 2005). However, the subset of robustly cyto c-labelled pyramidal neurons in neocortical layers III and V are described in the current study for the first time. These intensely labelled pyramidal neurons often occurred in clusters in layers III and V of the neocortex in all case types, and were independent of the presence of Aβ plaques. Similar clustering of pyramidal neurons labelled with NF triplet protein is observed in the cortex of monkeys, and may represent functional modules of neurons whose axons are bundled together (Peters and Sethares, 1996; Peters et al., 1997). Thus, cyto c-labelling may prove to be a useful neurochemical marker for layer V pyramidal neurons or a particular subset of clustered pyramidal neurons (Molnár and Cheung, 2006).

Punctate cellular cyto c-labelling was observed in a subset of cortical neurons including both intensely labelled pyramidal neurons and NFT-bearing neurons. Given that 62-74% of NFTs in the SFG are intracellular (Vickers et al., 2003), the cyto c-labelled neurons bearing NFTs represent a small minority of the total proportion of intracellular NFTs. Furthermore, the cyto c-labelling that co-localised with NFTs was punctate, indicating that NFT formation does not induce cyto c release and that NFTs may be only mildly detrimental to the health of NFT-bearing neurons. Thus, cyto c may label a subset of pyramidal neurons that demonstrate some capacity for NFT formation, but are relatively resistant to degeneration once NFT formation has initiated. Additionally, increased levels of cyto c are present in the neuronal soma and proximal processes following a variety of *in vivo* experimental insults (Martin and Liu, 2002; Benjelloun et

al., 2003; Page et al., 2003), suggesting that cyto c may be upregulated in compromised neurons as a sequela of AD associated pathology, possibly prior to NFT formation.

Cytochrome c immunolabelling was also frequently cytoplasmic within DNs in both preclinical AD and AD cases. Cytochrome c immunoreactivity is typically punctate and represents localisation to the mitochondria. Thus, evenly distributed cytoplasmic labelling may be indicative of cyto c release. Such cyto c release is present in CNS neurons *in vivo* following ischaemia, axotomy, contusion and traumatic axonal injury (Springer et al., 1999; Büki et al., 2000; Martin and Liu, 2002; Benjelloun et al., 2003; Cheung et al., 2003; Wingrave et al., 2003; Domañksa-Janik et al., 2004; Zhao et al., 2005). Additionally, the cyto c release in DNs indicates the possible activation of apoptotic pathways. Although Bax, aC8 and aC9 immunoreactivity are also present in DNs (MacGibbon et al., 1997; Nagy and Esiri, 1997; Tortosa et al., 1998; Rohn et al., 2002; Blanchard et al., 2003), aC3 has not been observed in DNs in AD cases to date, further supporting the proposition that activation of proteins with a role in apoptotic pathways does not necessarily translate into the execution of apoptosis (as discussed in Section 3.3). Furthermore, the presence of diffuse cyto c-labelling in DNs, but not in neuronal perikaryon in this study may imply discrete activation of apoptotic pathways that is reminiscent of the cyto c release observed in axonal segments, but not in the corresponding cell bodies, following axonal injury (Büki et al., 2000).

Many overlapping subsets of DNs are described that exhibit immunoreactivity for NF triplet proteins, α-internexin, APP, CgA and tau (Masliah et al., 1993; Su et al., 1996a; Vickers et al., 1996; Su et al., 1998; Dickson et al., 1999; Dickson et al., 2005). Differences in the complement of epitopes in DNs in preclinical AD cases compared to AD cases has lead to the suggestion that DNs mature; developing from APP, NF triplet protein- and α-internexin-labelled DNs into tau-labelled DNs (Su et al., 1996a; Su et al., 1998; Dickson et al., 1999; Dickson et al., 2005). In contrast to APP and NF triplet proteins, CgA is rarely co-localised with tau or NF triplet protein epitopes in DNs and may represent a form of reactive sprouting and synaptogenesis in these neurites (Dickson et al., 1999). Although cyto c-labelled DNs have previously been observed in

a subset of Aβ plaques in the human neocortex and the hippocampus and neocortex of various AD mouse models (Blanchard et al., 2003), there was no data on the co-localisation of cyto c with other DN markers. As NFM, CgA and tau label three predominant but overlapping subgroups of DNs (Su et al., 1998; Dickson et al., 1999), the co-localisation of these three DN markers and cyto c immunoreactivity was assessed.

Cytochrome c-labelling co-localised with a reasonably low percentage of tau-, NFM- and CgA-labelled DNs, indicating that cyto c was present within DNs at various stages of maturation, including the potentially reactively sprouting CgA-labelled DNs (Dickson et al., 1999). However, as cyto c immunoreactivity co-localised with a considerably lower percentage of tau-labelled DNs than with NFM- and CgA-labelled DNs, and a higher percentage of CgA- and NFM-labelled DNs were co-localised with cyto c in the preclinical AD cases compared to AD cases, cyto c-labelling may be a relatively early and transient alteration in DN maturation. Similarly, in transgenic mouse models of AD the density of cyto c-labelled DNs increases progressively with age until the later stages of disease progression, at which point the density of cyto c immunoreactive DNs decline (Blanchard et al., 2003). Continuing to study disease progression is crucial for attaining a better understanding of the pathological processes that occur in AD, and may lead to the discovery of novel therapeutic targets.

Thus, the punctate cyto c-labelling observed in a subset of neocortical neurons and NFTs suggests that cyto c may label a subset of neurons susceptible to NFT formation, but resistant to subsequent degeneration. Cytochrome c may well be a transient marker of early DNs, while the release of cyto c in a subset of DNs may be a sign of mitochondrial damage or dysfunction that could potentially activate apoptotic pathways.

5 β-AMYLOID PLAQUE-INDUCED AXONAL PATHOLOGY IN TRANSGENIC MICE EXPRESSING HUMAN MUTANT β-AMYLOID PRECURSOR PROTEIN GENES REPLICATES THE DYSTROPHIC NEURITE CHARACTERISTICS OF PRECLINICAL ALZHEIMER'S DISEASE

5.1 INTRODUCTION

Investigating the key pathological changes that occur as AD develops in order to identify primary causative factors has been difficult as postmortem human brain material provides only snap shots of disease progression. However, the use of transgenic mouse models of AD enables both potential therapeutics for AD, and aspects of age-associated disease progression to be investigated.

Transgenic mouse models of AD expressing human APP alone or in combination with PS1/PS2 harbouring mutations associated with familial AD, exhibit Aβ plaques and DNs, but not NFT pathology or extensive neuronal death (Games et al., 1995; Hsiao et al., 1996; Sturchler-Pierrat et al., 1997; Mucke et al., 2000; Chishti et al., 2001; Richards et al., 2003; Cheng et al., 2004). Tg2576 and TgCRND8 mice are two widely utilised mouse models of AD. Tg2576 mice exhibit Aβ plaque deposition beginning at seven months of age that progressively increases until severe pathology is present at 23 months of age (Hsiao et al., 1996; Kawarabayashi et al., 2001; Noda-Saita et al., 2004), while TgCRND8 demonstrate Aβ plaques at three months of age and DNs at five months of age (Chishti et al., 2001; Dudal et al., 2004).

The morphological and biochemical characteristics of DNs differ between preclinical AD and AD cases. Preclinical AD cases exhibit tortuous DNs as well as ring- and bulb-like DNs, which label with antibodies to NF triplet proteins and α-internexin, but are rarely labelled for abnormal tau protein (Cras et al., 1991; Benzing et al., 1993; Masliah et al., 1993; Su et al., 1996a; Vickers et al., 1996; Su et al., 1998; Dickson et al., 1999;

Dickson and Vickers, 2001; Dickson et al., 2005). Whereas, AD cases demonstrate bulb-like DNs that are immunolabelled for NF triplet proteins and α-internexin, numerous α-internexin immunoreactive ring-like DNs and classical elongated tau-labelled DNs (Dickson et al., 1999; Dickson et al., 2005).

Animal models are an invaluable tool for assessing the aging-related progression of diseases such as AD, and also for testing potential therapeutics for human disease. However, for animal models to be used effectively, a clear understanding of the pathological processes that they model is needed. In the current study, Aβ plaque-associated neuronal pathology in Tg2576 and TgCRND8 mice was investigated with a specific focus on the morphological and neurochemical phenotype of Aβ plaque-associated DNs. Specifically, the neuronal pathology associated with Aβ plaques in the transgenic AD mouse models was strikingly similar to that present in preclinical AD cases. The current investigation also indicates that the earliest Aβ plaque-associated neuronal alterations were the perturbation of NF triplet proteins and α-internexin in DNs. The results of this study suggest that transgenic AD mice represent an accurate and valuable model of the early pathological changes present in human AD.

5.2 MATERIALS AND METHODS

5.2.1 Tissue source and processing

As previously described (Section 2.1), human brain tissue was acquired from the Sun Health Research Institute and the National Health and Medical Research Council Brain Bank. Human cases include the ITG of seven AD cases (average age, age range; average postmortem interval, postmortem interval range: 79.5, 71-88 years; 7.5, 2.0-16.5 hours; Table 5.1) and the ITG of five preclinical AD cases (84.8, 78-91 years; 2.7, 2.3-3.0 hours; Table 5.1). There was no significant difference between the ages of the preclinical AD and AD cases, but the PMIs of the preclinical AD cases were significantly lower than those of the AD cases. It was not possible to gender match between case types due to the difficulty in obtaining precious human brain tissue. There was no robust association between sex and the percentage of neuritic plaques and there was no difference in DN phenotype between male and female cases, however, an interaction between sex and these two factors cannot be discounted nor could it be statistically analysed with this sample of cases.

A young cohort of 10 TgCRND8 mice expressing human APP695 harbouring the Swedish (KM670/671NL) and Indiana (V717F) mutations under the control of the Syrian hamster prion promoter on a hybrid C3H/He-C57BL/6 background (Chishti et al., 2001) and eleven age-matched wild type control mice were sacrificed at an early-pathology time point of 1.86-2.94 months of age. Similarly, an aged cohort consisting of eleven TgCRND8 mice and nine age-matched wild type controls were sacrificed at 7.5-8.91 months of age, a time point at which extensive pathology had developed. TgCRND8 mice were anaesthetised with 140mg/kg sodium pentobarbitone (Virbac, Peakhurst, Australia) and transcardially perfused (cold 0.01M PBS) and the left hemispheres were immersion-fixed in 4.0% paraformaldehyde. In addition, six 13 month-old Tg2576 mice expressing human APP695 harbouring the Swedish (KM670/671NL) familial AD mutation on a C57BL/6 and SJL hybrid background (Hsiao et al., 1996) and five age-matched wild type control mice were anaesthetised with sodium pentobarbitone and transcardially perfused (4% paraformaldehyde/0.01M PBS)

Table 5.1 Human cases used for immunohistochemistry and DN analysis.

Type	Age (years)	Gender	Postmortem interval (h)	Pathological Diagnosis
Preclinical AD	78	M	2.25	Post-operative
Preclinical AD	81	F	3	Cardiac arrest
Preclinical AD	84	M	3	Cardiopulmonary arrest
Preclinical AD	90	M	2.16	Respiratory arrest
Preclinical AD	91	M	3	Cardiac failure
AD	71	F	13	AD
AD	73	M	6.5	AD
AD	74	F	2	Pneumonia
AD	83	M	2.83	AD
AD	83	F	5	AD
AD	84	F	16.5	Cardiac failure
AD	88	M	7	Cardiac failure

at 13 months-of-age. Only a 13 month old cohort of Tg2576 mice was studied due to restrictions with mouse numbers, a time point at which substantial pathology is present in this mouse model (Kawarabayashi et al., 2001). The University of Tasmania Animal Research Ethics Committee approved all animal research included in this thesis. Following cryoprotection, (Section 2.1) 40μm coronal sections were cut on a cryostat.

5.2.2 Immunohistochemistry and analysis

To investigate the pathology present in the two transgenic mouse models of AD in comparison to human AD, the percentage of Aβ plaques that were neuritic was assessed. Thus, the ITG of five preclinical AD and five AD cases, all TgCRND8 and Tg2576 mice and their age-matched wild type control mouse cases were treated with formic acid (Section 2.3.1) and double labelled with antibodies to Aβ and α-internexin (Table 2.2). In addition, the aged TgCRND8 mice and human preclinical AD and AD cases were also stained with thioflavine s (Section 2.2.1) and labelled for α-internexin. Double labelling fluorescent immunohistochemistry was performed as previously described (Section 2.3.3), except that secondary fluorescent antibodies were used at a dilution of 1:1000. One hundred neocortical Aβ–labelled and 100 thioflavine s-stained plaques per case/animal were analysed for the presence of α-internexin-labelled dystrophic neurite clusters in the neocortex of aged TgCRND8 mice, and in the neocortex of the ITG of five human preclinical AD and five AD cases. The human ITG was selected for analysis of Aβ plaque deposition due to the abundance of plaques in this region in both preclinical AD and end-stage AD cases. The whole of the neocortex was used for mouse studies, as there was no predilection of Aβ plaques to particular cortical sub-regions in these animals, as compared to human cases. β-amyloid plaques were selected for analysis by examining non-overlapping vertical strips of neocortex from the pia to the white matter, up to a total of 100 plaques per case/animal. Analysis was performed for both Aβ immunolabelling and thioflavine s-staining as they label two subsets of plaques; specifically thioflavine s stains fibrillar aggregates within plaques, whereas the anti-Aβ antibody labels both fibrillar and non-fibrillar Aβ. Alpha-internexin was utilised as a DN marker for this analysis as α-internexin-labelled DNs are present in most DN

clusters (Dickson et al., 2005). No quantitative analysis was performed on the Tg2576 mouse tissue due to the low Aβ plaque load present. All quantitative data was analysed by a one-way ANOVA with a Bonferroni post-hoc analysis.

To compare the morphological and neurochemical characteristics of DNs in the transgenic AD mice with the human disease, the Tg2576 mice, aged TgCRND8 mice and human cases were also double labelled with combinations of mouse anti-α-internexin, -dephosphorylated NF triplet protein, -phosphorylated NF triplet protein, and -hyperphosphorylated-tau (phosphorylation at either Ser202/Thr205, Ser202/Ser205 or Ser205/Ser208) for axons, dendritic anti-MAP2 and rabbit anti-Aβ, as well as rabbit anti -α-internexin and -NFM antibodies (Table 2.2). Sections from the young cohort of TgCRND8 mice were also double labelled with α-internexin and dephosphorylated NF triplet protein. β-amyloid plaques were examined for the presence of bulb- and ring-like immunolabelled DNs throughout the neocortical laminae in the ITG of human cases, and throughout the neocortical laminae and hippocampus of the transgenic mice. With respect to neurofilament and α-internexin immunolabelling, bulb-like DNs are bulbar axonal swellings whereas ring-like DNs are spherical structures with an unlabelled hollow core (Dickson et al., 1999).

5.3 RESULTS

5.3.1 β-amyloid deposition and DN distribution in Tg2576 and TgCRND8 mice

As previously reported (Hsiao et al., 1996; Irizarry et al., 1997; Chishti et al., 2001; Kawarabayashi et al., 2001; Le et al., 2001; Tomidokoro et al., Dudal et al., 2004), both transgenic lines of AD mice exhibited Aβ plaques and DNs throughout the hippocampus, cortex, corpus callosum, and also in the striatum of TgCRND8 mice. Approximately 80-98% of Aβ plaques in aged TgCRND8 mice and Tg2576 mice were associated with clusters of dephosphorylated NF triplet protein-, phosphorylated NF triplet protein-, NFM-, and α-internexin-labelled DNs (Figure 5.1). The non-neuritic Aβ plaques in aged TgCRND8 mice were generally small and not fibrillar. In Tg2576 mice, few non-neuritic Aβ plaques were observed. There were also occasional clusters of NFM-, phosphorylated NF triplet protein-, and α-internexin-labelled DNs present in the absence of Aβ-labelling in aged TgCRND8 mice, but none were observed in Tg2576 mice.

Additionally, nine of the eleven young TgCRND8 mice demonstrated Aβ plaques, including a wide range of Aβ plaque deposition, with Aβ plaques being observed within the hippocampus and cerebral cortex, but rarely in the striatum. A few of the young TgCRND8 mice exhibited Aβ plaque deposition that was approximately equivalent to that in some 13 month old Tg2576 mice, however, the Aβ plaque deposition in 13 month old Tg2576 mice generally fell between that present in the young and aged TgCRND8 mice. Approximately 80-98% of Aβ plaques in the young TgCRND8 mice were associated with α-internexin- and dephosphorylated NF triplet protein-labelled DNs. None of the age-matched wild type controls for the Tg2576 or TgCRND8 mice exhibited Aβ immunoreactivity or DN clusters labelled for α-internexin.

All aged TgCRND8 mice demonstrated amyloid angiopathy, which was occasionally associated with thickened and bulb-like α-internexin-labelled DNs (Figure 5.2A).

Figure 5.1

Approximately 80-98% of Aβ plaques in both transgenic mouse models were associated with clusters of NFM-, and α-internexin-labelled DNs. Images of the TgCRND8 (**A**) and Tg2576 (**B**) neocortex double-labelled for Aβ (red, **A, B**) and α-internexin (green, **A**) or NFM (green, **B**) show numerous Aβ plaques co-localised with DN clusters (arrows). Some amyloid angiopathy (arrow heads) was also present that was not associated with DNs. Scale bar = 50μm.

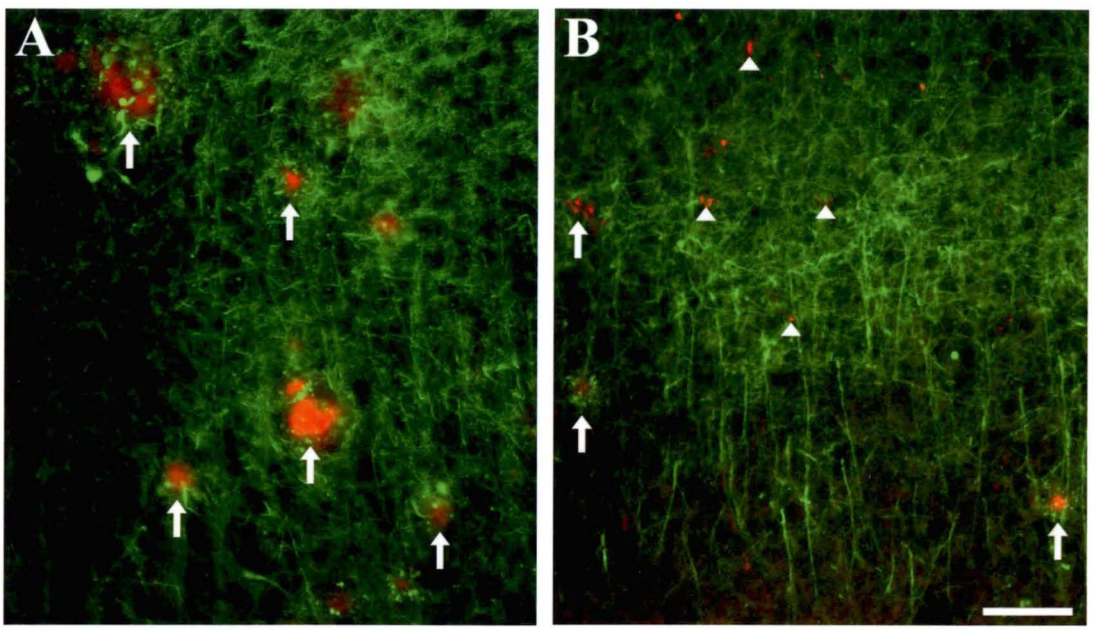
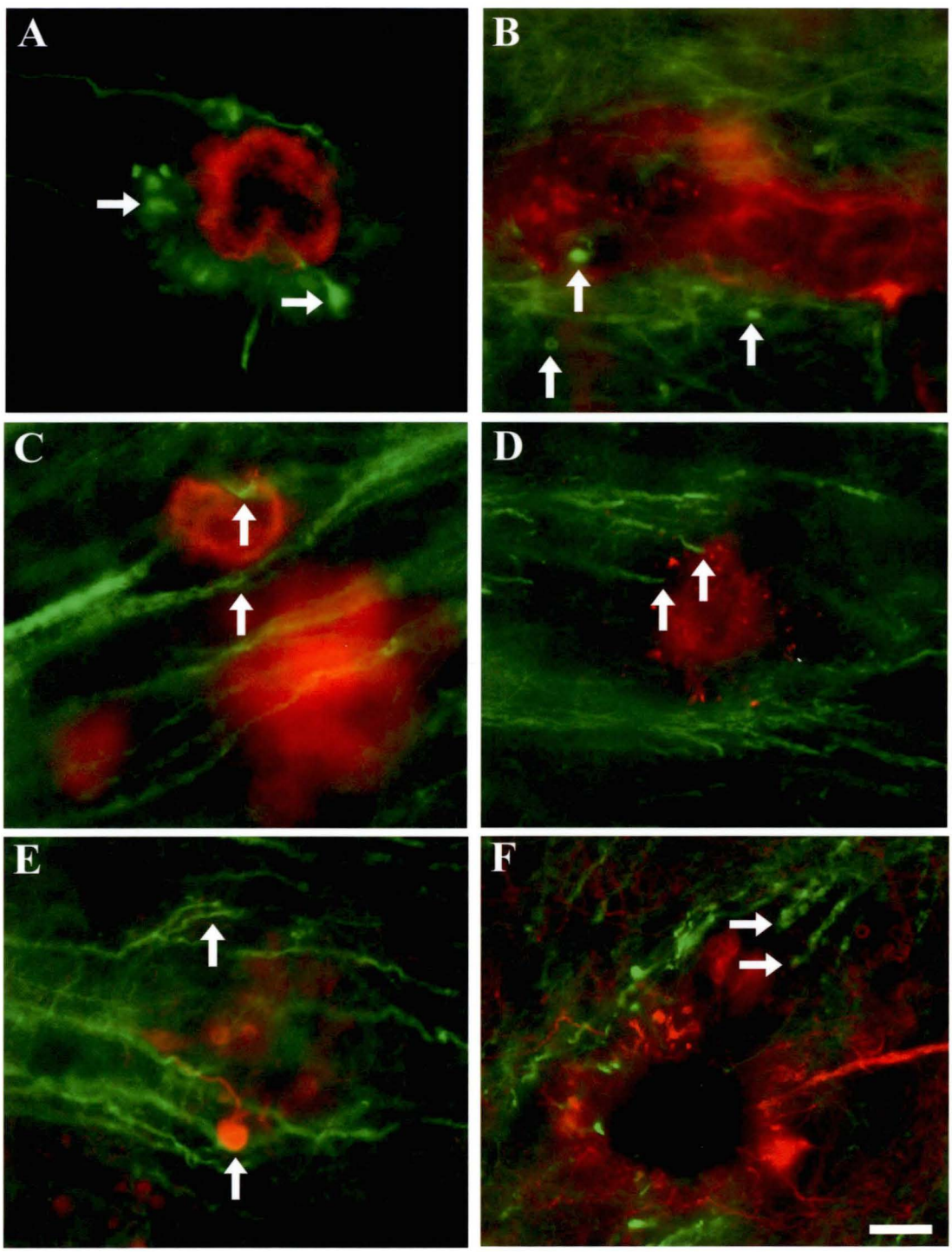

A
B

Figure 5.2

β-amyloid plaques caused neuropil disruption in both TgCRND8 and Tg2576 transgenic mice. Alpha-internexin-labelled DNs (green, arrows) were associated with β-amyloid-labelled amyloid angiopathy (red) in both aged TgCRND8 (**A**) and Tg2576 (**B**) mice. MAP2-labelled dendrites (green) appeared to bend (arrows) around Aβ plaques (red, **C**) and α-internexin-labelled DN clusters (red, **D**) in both Tg2576 (**C**) and TgCRND8 (**D**) mice. In addition, MAP-2-labelled dendrites (green) often appeared to terminate (arrows) at the margins of Aβ plaques (red, **E**) and α-internexin-labelled DN clusters (red, **F**) in both Tg2576 (**E**) and TgCRND8 (**F**) mice. Scale bar: A,B = 5μm, C-F = 10μm.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B
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D
E
F

Similarly, all Tg2576 mice exhibited amyloid angiopathy that was regularly associated with α-internexin-labelled DNs (Figure 5.2B). Additionally, consistent with previous confocal investigations in the neocortex of human AD cases (Adlard and Vickers, 2002), MAP2-labelled dendrites were also observed to deflect around or terminate at the margins of Aβ plaques in the Tg2576 mice and the aged TgCRND8 mice (Figure 5.2C-F).

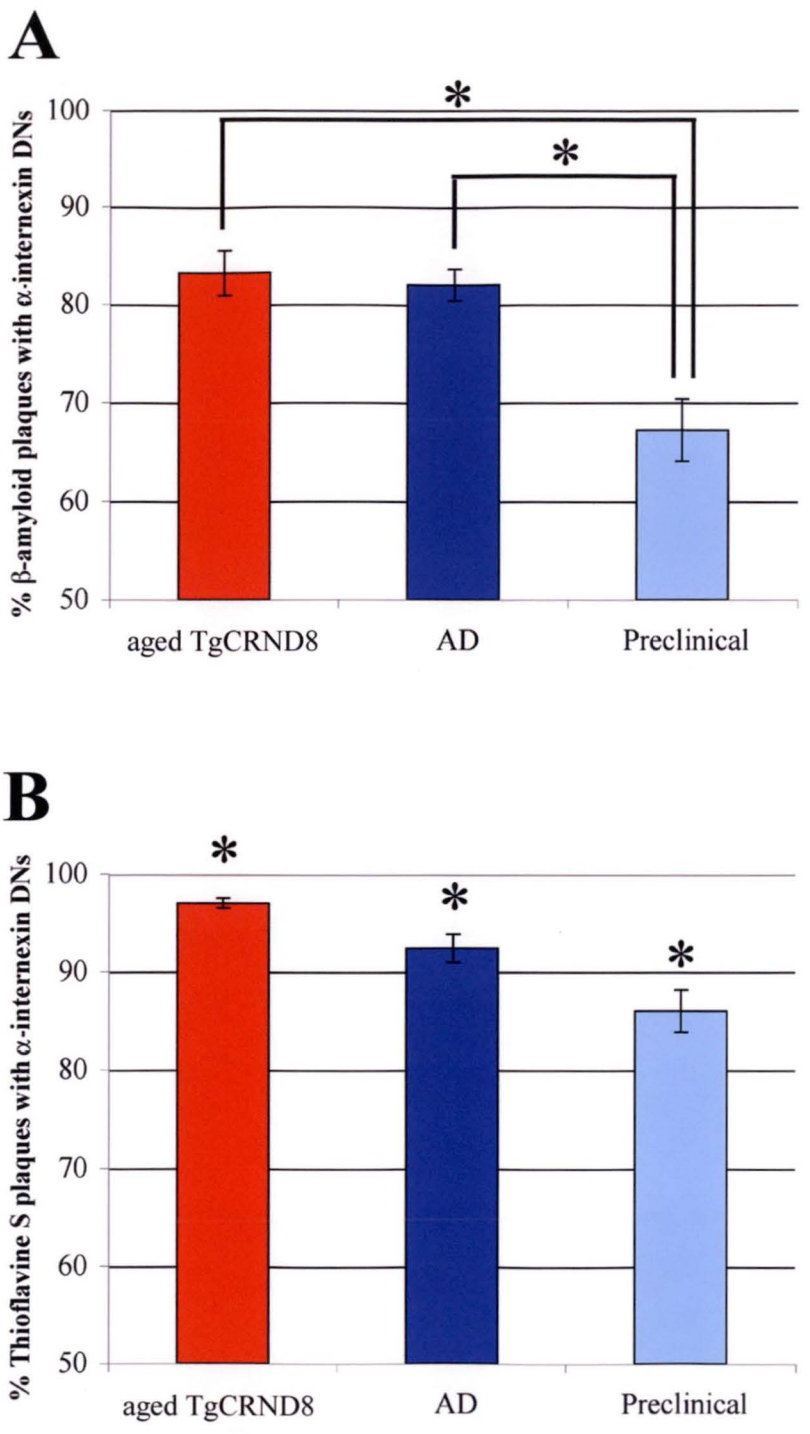
5.3.2 Percentage of neuritic Aβ plaques in TgCRND8 mice and human AD cases

To determine how disruptive Aβ plaques were to the axons (axonopathic) in the adjacent neuropil in transgenic AD mice relative to Aβ plaques in human preclinical AD and AD, the percentage of Aβ plaques associated with α-internexin-labelled DNs was assessed in the cortex of aged TgCRND8 and the ITG of human preclinical AD and AD cases. The percentage of Aβ plaques associated with α-internexin-labelled DNs in aged TgCRND8 mice was similar to the percentage of neuritic Aβ plaques present in human AD cases, but significantly different from the percentage of neuritic Aβ plaques observed in preclinical AD cases (Figure 5.3A; $p < 0.05$). In the aged TgCRND8 mice, 83.2 ± 2.3% (average ± SEM) of Aβ plaques were associated with α-internexin-labelled DNs, while the percentage of Aβ plaques associated with α-internexin-labelled DNs in human AD and preclinical AD cases were 82.0 ± 1.6% and 67.2 ± 3.2%, respectively.

In contrast, the percentage of fibrillar thioflavine s-stained plaques associated with α-internexin-labelled DNs in the aged TgCRND8 mice and human preclinical AD and AD cases were all significantly different (Figure 5.3B; $p < 0.05$). In aged TgCRND8 mice, the percentage of thioflavine s-stained plaques that co-localised with α-internexin-labelled DNs was 97.0 ± 0.5%. Whereas, in human AD and preclinical AD cases, the percentage of thioflavine s-stained plaques associated with α-internexin-labelled DNs were 92.4 ± 1.4% and 86.0 ± 2.2%, respectively.

Figure 5.3

β-amyloid-labelled and thioflavine s-stained plaques were highly axonopathic in aged TgCRND8 mice. The percentage of Aβ-labelled (**A**) and thioflavine s-stained (**B**) plaques in aged TgCRND8 mice that were associated with α-internexin-labelled DNs was significantly higher than the percentage of neuritic plaque present in human preclinical AD. The percentage of thioflavine s-stained plaques associated with α-internexin-labelled DNs in aged TgCRND8 mice was significantly higher than that present in AD. Error bars indicate SEM. * $p < 0.05$


A
% β-amyloid plaques with α-internexin DNs
100
90
80
70
60
50
*
*
aged TgCRND8
AD
Preclinical
B
% Thioflavine S plaques with α-internexin DNs
100
90
80
70
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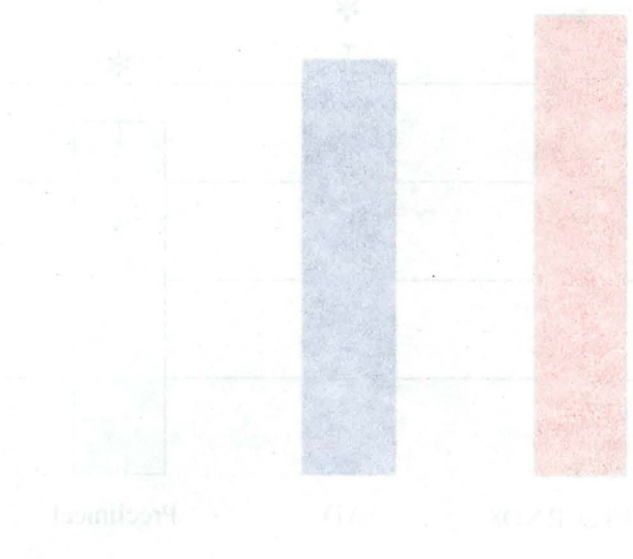
*
*
aged TgCRND8
AD
Preclinical



5.3.3 Morphology and neurochemistry of DNs

Dystrophic neurites have been categorised as aberrant tortuous neurites with variable diameters, and as ring- and bulb-like based on the morphology of the labelled elements (Benzing et al., 1993; Masliah et al., 1993; Su et al., 1996a; Vickers et al., 1996; Su et al., 1998; Dickson et al., 1999; Dickson and Vickers, 2001; Dickson et al., 2005). Both Tg2576 and aged TgCRND8 mice exhibited dephosphorylated NF triplet protein-, phosphorylated NF triplet protein-, α-internexin- and NFM-labelled ring- and bulb-like DNs (Figure 5.4), along with Aβ plaque-associated non-classical punctate and fine-thread-like hyperphosphorylated-tau-labelling (Figure 5.5B,C). Interestingly, although the young cohort of TgCRND8 mice demonstrated dephosphorylated NF triplet protein- and α-internexin-labelled DNs, no hyperphosphorylated-tau-labelling was associated with Aβ plaques at this early pathology time point (Figure 5.5A). In comparison, human preclinical AD cases exhibited α-internexin-, dephosphorylated NF triplet protein-, phosphorylated NF triplet protein- and NFM-labelled ring- and bulb-like DNs (Figure 5.4), and Aβ plaque-associated punctate and fine-thread-like hyperphosphorylated-tau-labelling (Figure 5.5D). Although, occasional classical hyperphosphorylated-tau DNs were present in a subset of preclinical AD cases. In contrast, the human AD cases exhibited abundant classical hyperphosphorylated-tau-labelled DNs (Figure 5.5), numerous α-internexin, phosphorylated NF triplet protein-, dephosphorylated NF triplet protein- and NFM-labelled bulbous DNs, along with numerous α-internexin-labelled, but only rare NF triplet protein-labelled ring-like DNs (Figure 5.4).

Hyperphosphorylated-tau-labelling was not specifically co-localised with NFM- or α-internexin-labelled DNs in both TgCRND8 and Tg2576 mice (Figure 5.6A-F). However, labelling for dephosphorylated and phosphorylated NF triplet proteins often co-localised with labelling for NFM (phosphorylation independent) and/or α-internexin within DNs in both transgenic mouse models (Figure 5.6G-L). In addition, hyperphosphorylated-tau-labelled DNs were observed to co-localise with α-internexin- and NFM-labelled DNs in AD cases (Dickson et al., 1999; Dickson et al., 2005).

Figure 5.4

The DN pathology present in TgCRND8 mice (**A-D**) and Tg2576 mice (**E-H**) was identical to that observed in human preclinical AD (**I-L**), but not AD (**M-P**). Representative images were acquired after double immunohistochemistry for Aβ (**A-P**) with α-internexin (**A, E, I, M**), NFM, (**B, F, J, N**) dephosphorylated NF triplet protein (**C, G, K, O**) or phosphorylated NF triplet protein (**D, H, L, P**). β-amyloid plaques (red) were associated with numerous bulb- (arrows) and ring-like (arrow heads) α-internexin-, NFM-, dephosphorylated NF triplet protein- and phosphorylated NF triplet protein-labelled DNs (green) in both transgenic mouse models and human preclinical AD. However, in human AD Aβ plaques were associated with abundant bulb-like DNs, and only α-internexin-labelled ring-like DNs. Scale bar = 10μm.

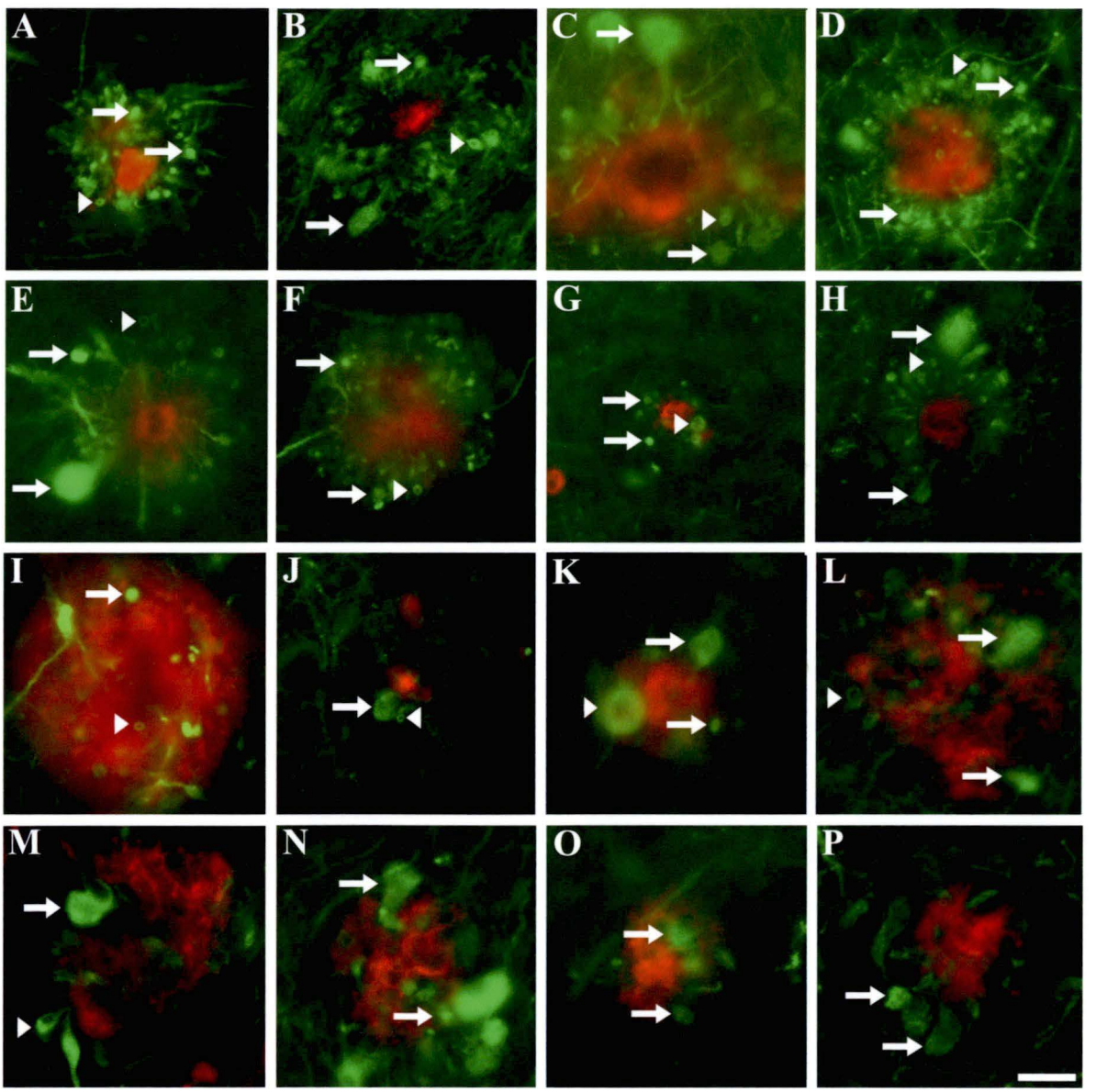

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Figure 5.5

Abundant classical elongated hyperphosphorylated-tau-labelled DNs were observed in human AD, but not human preclinical AD cases or transgenic AD mice. No hyperphosphorylated-tau-labelling (green) was observed in association with Aβ plaques (red) in the young TgCRND8 mice (**A**). Punctate (arrows) and fine thread-like (arrow head) labelling for hyperphosphorylated-tau (green) were observed in associated with Aβ plaques (red) in aged TgCRND8 (**B**) and Tg2576 (**C**) mice. While human preclinical AD cases (**D**) exhibited punctate (arrows), fine thread-like (arrow head) and hyperphosphorylated-tau-labelling (green) associated with Aβ plaques (red), and occasional classic elongated hyperphosphorylated-tau-labelled DNs (large arrow head). Numerous classic angular and elongated (large arrow heads) hyperphosphorylated-tau-labelled DNs (green) were present in association with Aβ plaques (red) in human AD (**E**). In human AD cases (**F**) α-internexin-labelled (red) and hyperphosphorylated-tau-labelled (green) DNs co-localised (arrows). Scale bar = 10μm.

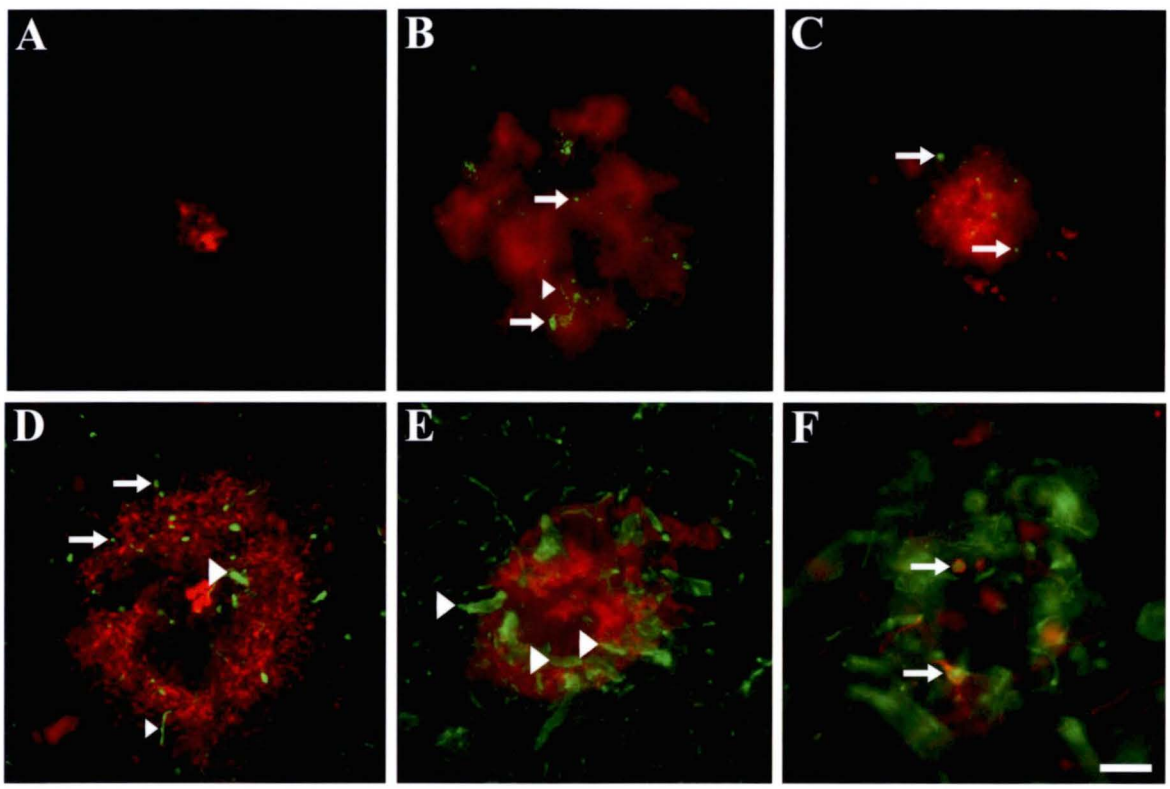
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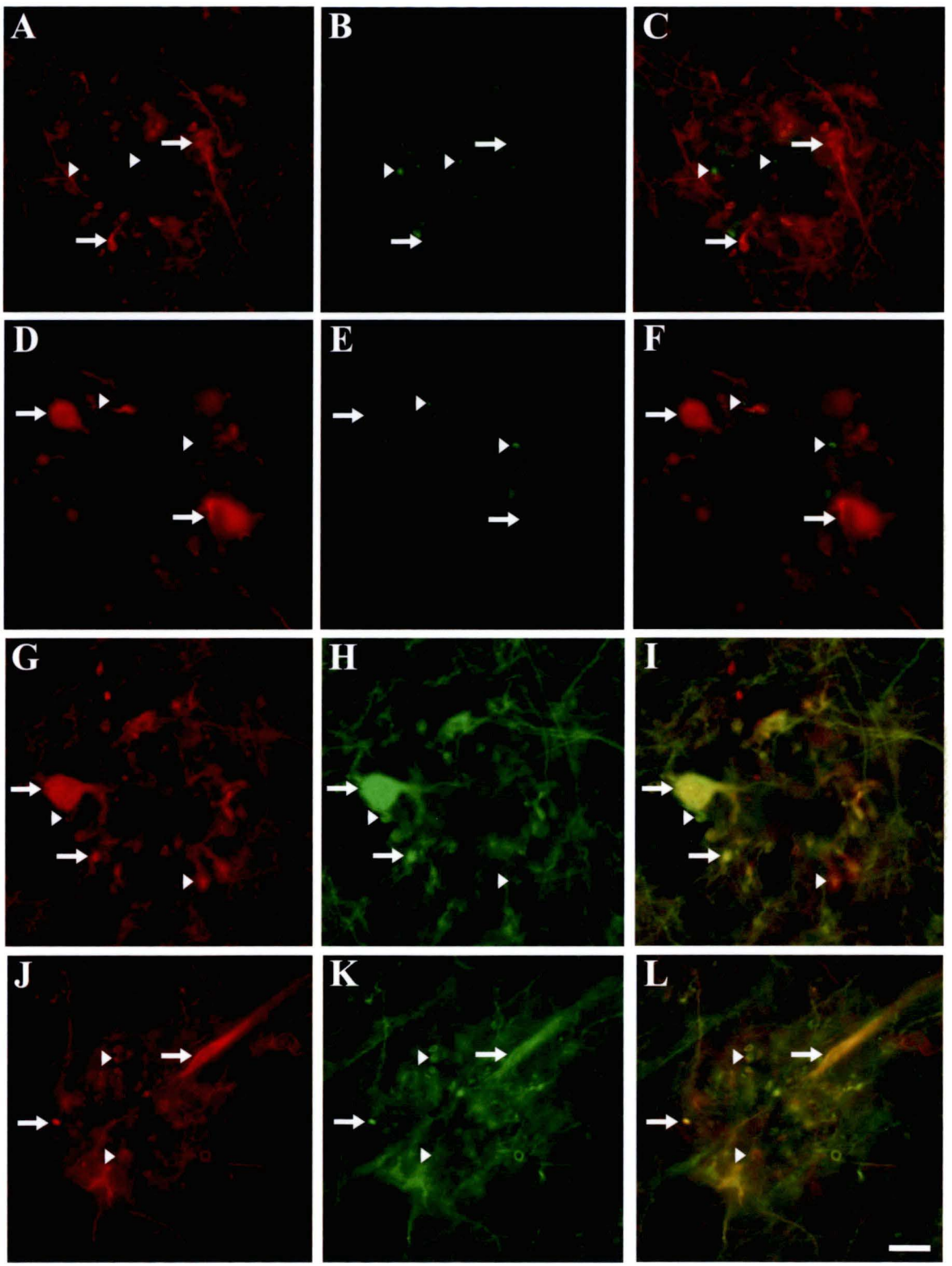
Figure 5.6

Hyperphosphorylated-tau-labelling did not specifically co-localise with α-internexin- or NFM-labelled DNs. When images of α-internexin-labelled (**A, D**, arrows, red) DNs and punctate and fine-thread-like hyperphosphorylated-tau-labelling (**B, E**, arrow heads, green) in aged TgCRND8 mice (**A-C**) and Tg2576 mice (**D-F**) were overlayed (**C, F**) no specific co-localisation between these two subsets of DNs was observed. In comparison, when images of α-internexin-labelled DNs (**G, J**, red) and phosphorylated NF triplet protein-(**H**, green) or NFM-labelled (**K**, green) DNs in TgCRND8 (**G-I**) and Tg2576 (**J-L**) mice were overlayed (**I, L**), the cytoskeletal markers co-localised within the majority of DNs (arrows), although some DNs were immunolabelled with α-internexin, phosphorylated NF triplet protein or NFM alone (arrow heads). Scale bar = 10μm.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5.4 DISCUSSION

Notably, the current study has shown that two widely utilised transgenic AD mouse models exhibit DN pathology that is morphologically and neurochemically identical to that of human preclinical AD cases, but not AD cases. β-amyloid plaque deposition, as well as amyloid angiopathy, was observed to disrupt the surrounding neuropil in both TgCRND8 and Tg2576 mice. Specifically, Aβ plaque formation was highly axonopathic in aged TgCRND8 mice, and dendrites adjacent to Aβ plaques were also disrupted in both transgenic mouse models. Additionally, as Aβ plaque-associated hyperphosphorylated-tau-labelling was observed in the aged TgCRND8 mice, but not in the young cohort of TgCNRD8 mice, the current study supports the proposition that the phenotype of DNs matures as AD progresses from NF triplet protein and α-internexin-abundant forms through to hyperphosphorylated-tau-labelled DNs.

Both amyloid angiopathy and Aβ plaques were associated with abnormal neuronal processes in both transgenic mouse models, suggesting that Aβ plaque deposition locally disrupts adjacent neuropil. Amyloid angiopathy was observed in association with α-internexin-labelled DN structures in both Tg2576 and TgCRND8 mice. In addition, Aβ plaques caused both dendritic and axonal disruption, although the reaction of axons and dendrites to damage was different. Dystrophic neurites of axonal origin have been observed in association with Aβ plaques in Tg2576 and TgCRND8 mice in this, and previous studies (Irizarry et al., 1997; Chishti et al., 2001; Tomidokoro et al., 2001; Motoi et al., 2004; Noda-Saita et al., 2004; Delatour et al., 2004). Interestingly, anterograde tracer studies in transgenic AD mice that express human mutant APP and PS1 describes similar axonal pathology largely restricted to cortico-cortical connections (Delatour et al., 2004) indicating a specific disruptive and localised effect of Aβ plaque formation. In addition, MAP2-labelled dendrites were observed to "bend" around Aβ plaques and were also "clipped" at Aβ plaque margins in the TgCRND8 and Tg2576 mice. Similarly, Aβ plaques also affect the structure and organisation of adjacent dendrites in human AD (Knowles et al., 1998; Adlard and Vickers, 2002; Grutzendler et al., 2007) and other transgenic AD mouse models (Le et al., 2001; Schwab et al., 2004;

Grutzendler et al., 2007), supporting the idea that Aβ plaque formation may have a localised compressing effect on the neuropil.

The percentage of neuritic Aβ-labelled plaques in aged TgCRND8 mice was not significantly different from that in AD cases, while the percentage of neuritic thioflavine s-stained plaques was significantly higher in aged TgCRND8 mice than in AD cases. Additionally, the vast majority of Aβ plaques in aged TgCRND8 mice and Tg2576 mice were observed to be associated with dephosphorylated NF triplet protein-, phosphorylated NF triplet protein- and NFM-labelled DNs. These data suggest that the Aβ plaques present in aged TgCRND8 mice are highly axonopathic relative to human preclinical AD and AD cases. The current data also corroborates previous reports (Dickson and Vickers, 2001), of the percentage of neuritic Aβ plaques in human AD being significantly higher than the percentage of neuritic Aβ plaques in preclinical AD cases (Figure 5.3A; $p < 0.05$). Furthermore, a higher percentage of thioflavine s-stained amyloid plaques were associated with DNs compared to Aβ-labelled plaques for aged TgCRND8, AD and preclinical AD cases. These data support previous *in vitro* reports of fibrillar Aβ being more detrimental to cell health than amorphous Aβ (Pike et al., 1993; Lorenzo and Yanker, 1994; Ivins et al., 1998), and evidence from *in vivo* studies that fibrillar Aβ plaques are associated with increased damage to the surrounding neuropil in comparison to diffuse Aβ plaques (Dickson et al., 1988; Fukumoto et al., 1996; Su et al., 1996a; Knowles et al., 1998; Dickson and Vickers, 2001; Le et al., 2001; Adlard and Vickers, 2002; D'Amore et al., 2003; Noda-Saita et al., 2004; Brendza et al., 2005; Dickson et al., 2005).

Dystrophic neurites labelled for tau (with variable phosphorylation), NF triplet proteins, MAP2, APP, PS1, synaptophysin, α-synuclein and ubiquitin are present in various transgenic mouse models of AD (Games et al., 1995; Masliah et al., 1996b; Irizarry et al., 1997; Sturchler-Pierrat et al, 1997; Chishti et al., 2001; Rockenstein et al., 2001 Tomidokoro et al., 2001; Diez et al., 2003; Kurt et al., 2003; Boutajangout et al., 2004; Bussière et al., 2004; Motoi et al., 2004; Noda-Saita et al., 2004 Schwab et al., 2004; Bellucci et al., 2007). However, the current investigation is the first to report that the

DN pathology in transgenic AD mice was morphologically and neurochemically identical to the DNs in human preclinical AD, but not AD (Table 5.2).

The presence of abundant NF triplet protein and α-internexin-labelled ring- and bulb-like DNs and the lack of classical phosphorylated tau-labelled DNs in Tg2576 and TgCRND8 mice is characteristic of human preclinical AD, but not AD cases (Table 5.2). In contrast, human AD cases demonstrate numerous angular and elongated tau-labelled DNs, but only rare Aβ plaque-associated NF triplet protein-labelled ring-like DNs (Dickson et al., 1999; Dickson et al., 2005; Table 5.2). Conversely, the transgenic AD mice exhibited punctate and fine thread-like hyperphosphorylated-tau-labelling that was strikingly similar to the punctate and fine thread-like hyperphosphorylated-tau-labelling in human preclinical AD (Table 5.2). However, it should be noted that a subset of preclinical AD cases do demonstrate occasional hyperphosphorylated-tau DNs with classical elongated morphology.

The evidence that the DN pathology present in Tg2576 and TgCRND8 mice resembles human preclinical AD more closely than AD cases has important implications for the experimental use of these, and perhaps other, mouse models. While an increasing number of studies utilise transgenic mouse models of AD for testing potential therapeutic agents (Schenk et al., 1999; Janus et al., 2000; Sigurdsson et al., 2001; Leissring et al., 2003; Bergamaschini et al., 2004; Chauhan et al., 2004; Arbel et al., 2005; Singer and Marr, 2005; Asai et al., 2006; McLaurin et al., 2006), the beneficial effects seen in such animal models have not always translated to the human condition. For example immunotherapy treatment that was effective in several transgenic mouse models of AD, proved to be detrimental to the health of subjects in human Phase IIA clinical trials (Orgogozo et al., 2003). This may be because such therapeutics are able to efficiently reverse the early pathological changes in AD (Oddo et al., 2004), such as those present in transgenic mouse models of AD and human preclinical AD, but may be inefficient for treating AD in which neurofibrillary pathology is well established and overt neuronal loss has already occurred (Vickers et al., 2000; Nicoll et al., 2003; Ferrer et al., 2004). In support of this proposition, Oddo and colleagues (2004) discovered that

Table 5.2 Summary of the DN types present in aged TgCRND8 and Tg2576 transgenic mice, and human preclinical AD and AD cases.

Dystrophic neurite type	Transgenic AD mice	Preclinical AD	AD
Bulb-like NF triplet protein-labelled	+	+	+
Bulb-like α-internexin-labelled	+	+	+
Ring-like α-internexin-labelled	+	+	+
Ring-like NF triplet protein-labelled	+	+	-
Elongated hyperphosphorylated-tau-labelled	-	-	+

Aβ immunotherapy could reverse the early, but not the late stages of pathological tau hyperphosphorylation in a transgenic AD mouse model. In addition, two human AD subjects that received Aβ immunotherapy exhibited extensive neocortical areas with very few Aβ plaques and DNs, but no reduction of NFTs or neuropil threads (Nicoll et al., 2003; Ferrer et al., 2004). This is particularly important, as clinical trials for potential therapeutics involve human AD subjects, and there is currently no effective way to diagnose preclinical AD in humans. However, the accurate diagnosis of early AD cases prior to a clinical diagnosis utilising a combined biomarker/MRI imaging approach may soon be possible (as reviewed in Borroni et al., 2007; de Leon et al., 2007).

Aged TgCRND8 mice demonstrated α-internexin- and NF triplet protein-labelled DNs and hyperphosphorylated-tau-labelling, conversely the young cohort of TgCRND8 mice exhibited α-internexin- and NF triplet protein-labelled DNs, but no hyperphosphorylated-tau-labelling. Neurofilament-labelled DNs are also observed several months before phosphorylated tau-labelled DNs in TgCRND8 and other transgenic AD mouse models (Masliah et al., 2001; Blanchard et al., 2003; Boutajangout et al., 2004; Bellucci et al., 2007). These data suggest that NF triplet protein- and α-internexin-labelled DNs may be the earliest neuritic pathology to appear in association with Aβ plaques, while the presence of phosphorylated tau in DNs occurs at a later time point (Su et al., 1998; Dickson et al., 1999; Masliah et al., 2001; Dickson et al., 2005; Bellucci et al., 2007). Similarly, it has been proposed that in human AD, DNs may first appear in preclinical AD labelled for NF triplet proteins and α-internexin, then develop a core of altered tau filaments before they become the elongated tau-only DNs observed in AD (Su et al., 1998; Dickson et al., 1999; Dickson et al., 2005). Additionally, these findings also suggest that tau hyperphosphorylation occurs as a secondary event to Aβ plaque deposition, which is supported by evidence that the presence of Aβ results in an increase in tau hyperphosphorylation *in vitro* (Busciglio et al., 1995; Ferreira et al., 1997; De Felice et al., 2007).

The hyperphosphorylated-tau antibody used in this study detects the hyperphosphorylation of tau at either Ser202/Thr205, Ser202/Ser205 or Ser205/Ser208

(Porzig et al., 2007). In particular, hyperphosphorylation of tau at Ser202 is a relatively early phosphorylation event in AD tau pathology and is an epitope associated with the presence of abnormal PHF-tau (Braak et al., 1994; Su et al., 1994b). In human AD this abnormal PHF-tau is present in both DNs and somatic NFTs. Thus, it has been postulated that the punctate and fine-thread-like hyperphosphorylated-tau-labelling in transgenic mice may be equivalent to the PHF-tau-labelled DNs of human AD, but that mice do not have a long enough lifespan to allow NFTs to develop (Masliah et al., 2001; Kurt et al., 2003). However, although DNs that exhibit phosphorylated tau epitopes are present in transgenic AD mice, no PHFs have been observed at the ultrastructural level in DNs in these animals (Lewis et al., 2001; Masliah et al., 2001; Sasaki et al., 2002; Kurt et al., 2003), despite mouse tau being almost homologous to human tau. The lack of PHFs in DNs in transgenic mouse models despite the presence of PHF-associated hyperphosphorylated-tau epitopes supports the proposition that transgenic AD mice represent an incomplete model of AD, but more comparably model early or preclinical AD (Phinney et al., 1999; Masliah et al., 2001; Vickers et al., 2001; Schwab et al., 2004).

In summary, the Aβ plaque-associated neuritic pathology present in Tg2576 and TgCRND8 mice was morphologically and biochemically identical to the cytoskeletal pathology of DNs present in preclinical AD, but not AD. Thus, these mice provide important opportunities to examine the early sequence of cellular changes that lead to the development of AD pathology, and the mechanisms by which the pathological process progresses and affects cellular function and health. Moreover, transgenic AD mouse models will serve as a vital platform for examining new therapeutic approaches that can be administered before extensive neuronal degeneration occurs.

6 METALLOTHIONEIN ISOFORM IIA AS A POTENTIAL THERAPEUTIC FOR THE TREATMENT OF ALZHEIMER'S DISEASE

6.1 INTRODUCTION

Currently, AD patients are commonly treated with cholinesterase inhibitors (Brion, 1996; Brodaty et al., 2001) or NMDA glutamate receptor antagonists (Livingston and Katona, 2004), but these therapeutics are only effective in a subset of AD patients, and do not affect AD progression (Parnetti et al., 1997). Thus, many research groups are searching for more effective therapeutic interventions for AD, and numerous potential therapeutic agents are currently in human clinical trials (Ruther et al., 2000; Simons et al., 2002; Jiang et al., 2003; Ritchie et al., 2003; Dodel et al., 2004; Sparks et al., 2005; Siemers et al., 2006). Assuming that Aβ plaques are the primary causative agent of AD, there are two potential approaches to slow or prevent AD: inhibit or stop Aβ plaque formation by modulating APP processing, inhibiting Aβ aggregation or increasing Aβ clearance, or protect neurons against the damaging effects of Aβ with anti-inflammatory drugs, antioxidants or growth factors (Vickers et al., 2000). Thus, an effective treatment strategy for AD would be to simultaneously inhibit Aβ plaque formation and to protect neurons against Aβ induced damage. Indeed, an increasing number of therapeutic agents with multiple modes of action are currently being investigated including NSAIDs, atorvastatin, verivastatin, fluvastatin, lovastatin, pravastatin, simvastatin, cerebrolysin and Huperzine A (Weggen et al., 2001; Simons et al., 2002; Sjogren et al., 2003; Zhang et al., 2004b; Rockenstein et al., 2006; Wang et al., 2006).

One such potential therapeutic agent for treating AD is metallothionein (MT) isoforms I and II (MTI/II), which has neuroprotective and antioxidant properties, and may also inhibit Aβ aggregation through its metal chelating ability (Maret, 1995; Aschner et al., 1997; Adlard et al., 1998; Penkowa et al., 1999; Giralt et al., 2002; Chung et al., 2003). Metallothioneins are small (6-7 kDa), cysteine rich proteins that are present in most cells in the body including some cell types in the CNS (Hidalgo et al., 2001). Metallothionein isoform III (MTIII) is a growth inhibitory MT isoform that is present in neurons within

the hippocampus, amygdala and cerebellum in the healthy brain (Masters et al., 1994), but there are contradictory reports of MTIII expression in AD brains, including downregulation (Uchida et al., 1991; Yu et al., 2001), no change (Amoureux et al., 1997) and upregulation of MTIII (Carrasco et al., 1999). Metallothionein isoforms I and II are expressed throughout the brain, predominantly by astrocytes, with a vast body of evidence demonstrating that MTI/II protects the brain against damage, excess heavy metals and oxidative and inflammatory stress (reviewed in Hidalgo et al., 2001). In addition, MTI/II are significantly upregulated in several transgenic mouse models of AD (Carrasco et al., 2006) and in human AD (Duguid et al., 1989; Adlard et al., 1998; Zambenedetti et al., 1998). Metallothioneins were considered to be strictly intracellular proteins, but recent studies have demonstrated an extracellular role for MTI/II (reviewed in Chung and West, 2004). Administration of exogenous MTI/II is highly efficacious in animal models of CNS injury, multiple sclerosis and Parkinson's disease (Giralt et al., 2002; Chung et al., 2003; Penkowa and Hidalgo, 2003; Xie et al, 2004).

Metallothionein isoforms I and II have a neuroprotective role following axonal injury *in vitro* and several types of CNS injury *in vivo* (Penkowa et al., 1999; Giralt et al., 2002; Chung et al., 2003; Chung and West, 2004). Additionally, MTI/II null mice exhibit increased damage and poor recovery following physical or inflammatory injury to the CNS when compared to mice with endogenous MT (Penkowa et al., 1999; Giralt et al., 2002). In this respect, the upregulation of MTI/II in AD may, in part, be due to the localised mechanical injury caused by Aβ plaques. Metallothioneins are also powerful chelators and have a role in sequestering heavy metals and regulating the availability of metals for enzymes and transcription factors (Adlard et al., 1998). Each MT molecule is able to bind seven Zn^{2+} or 10 Cu^{+} metal ions, and through its metal binding properties MT may maintain metal homeostasis. Metal homeostasis is altered in AD with increased levels of extracellular metals and decreased bioavailability of metals within the cell, which is consistent with the dysregulation of APP processing, increased tau hyperphosphorylation, Aβ aggregation and increased oxidative stress present in AD (as reviewed in Adlard and Bush, 2006; Crouch et al., 2007). In addition, MTI/II may influence a wide variety of cellular processes via its metal chelating abilities, including

Aβ aggregation and Aβ associated free radical production. β-amyloid has copper and zinc binding sites that affect the reversible precipitation of Aβ into insoluble masses (Bush et al., 1994; Huang et al., 1997; Atwood et al., 1998) and the production of hydrogen peroxide that is mediated by redox-active metal ion and Aβ interactions (Cherny et al., 1999; Huang et al., 1999; Opazo et al., 2002). Regardless of the mechanism, free radicals are increased in AD brains (Smith et al., 1996; Smith et al., 1997). Thus, by preventing the accumulation of redox active metals and free radical-mediated cellular damage, MTI/II may have an important role as an antioxidant (Maret, 1995; Aschner et al., 1997). Indeed, in mouse models of amyotrophic lateral sclerosis and multiple sclerosis (experimental autoimmune encephalomyelitis) MTI/II deficiency resulted in increased oxidative stress and disease symptoms (Nangano et al., 2001; Puttaparthi et al., 2002; Penkowa et al., 2003).

In light of the success of MTI/II administration to animal models of CNS injury and neurodegenerative diseases, and the biology of MTI/II as a free radical scavenger, zinc and copper sink and neurotrophic factor, a pilot study for the treatment of transgenic Tg2576 AD mice with exogenous MTIIA was undertaken.

6.2 MATERIALS AND METHODS

6.2.1 Metallothionein IIA administration

Nine Tg2576 mice expressing human APP695 harbouring the Swedish (KM670/671NL) familial AD mutation on a C57BL/6 and SJL hybrid background (Hsiao et al., 1996) and five age-matched wild type mice were injected intraperitoneally with 1μg of sterile Zn-MTIIA/g body weight (7.0 mol Zn equivalent; Bestenbalt LLC, Tallinn, Estonia) three times a week for 12 weeks beginning at 10 months of age. A dose of 1μg of sterile Zn-MTIIA/g body weight was chosen as previous studies have chronically injected mice with MTIIA intraperitonaelly with this dose daily for a maximum of seven days and observed neuroprotection following focal brain injury (Giralt et al., 2002). A higher dose of MTIIA was not utilised as previously published studies have only administered MTIIA daily for a maximum of seven days in mice (Giralt et al., 2002) and any possible detrimental effects of chronic administration of higher doses of MTIIA have yet to be determined. MTIIA has been successfully administered by intraperitoneal injection in previous studies that involved animal models of CNS disease or injury with definite or potential disruption of the BBB (Giralt et al., 2002; Penkowa and Hidalgo, 2003; Xie et al., 2004). Thus, MTIIA was administered intraperitoneally in Tg2576 mice as the BBB is compromised in these mice (Ujiie et al., 2003; Kumar-Singh et al., 2005; Dickenstein et al., 2006). Tg2576 mice were utilised for this pilot study as they are a widely utilised transgenic AD mouse model that have been used to test many potential therapeutic compounds (Weggen et al., 2001; Sung et al., 2004; Rezai-Zadeh et al., 2005; Hartman et al., 2006; Li et al., 2006). Metallothionein isoform IIA was chosen for this study as it has been shown to be efficacious in animal models of CNS injury and previously published literature indicates that MT-II functions are unlikely to be different from those of MT-I (Giralt et al., 2002; Chung et al., 2003; Penkowa and Hidalgo, 2003; Xie et al, 2004). Metallothionein isoform IIA protein was administered as the zinc thionein form as mammalian MTs are predominantly bound to zinc *in vivo* (Vasak, 2005). Six control Tg2576 mice and five age-matched wild type mice were also injected intraperitoneally

with 20µl of sterile 0.01M PBS three times a week for 12 weeks starting at 10 months of age.

To investigate whether a higher Aβ plaque load at the end point of the treatment regimes would more clearly delineate the potential therapeutic effects of MTIIA, a second cohort of older Tg2576 mice were also treated. Six Tg2576 mice and five age-matched wild type mice were injected intraperitoneally with 1µg of sterile Zn-MTIIA/g body weight three times a week for 12 weeks beginning at twelve months of age. Four control Tg2576 mice and five age-matched wild type mice were also injected intraperitoneally with 20µl of sterile 0.01M PBS three times a week for 12 weeks starting at 12 months of age. All mice treated were weighed at the beginning, middle and end of the treatment program.

6.2.2 Tissue processing

At the end of the treatment regimes, Tg2576 and wild type control mice were anaesthetised with 140mg/kg sodium pentobarbitone and transcardially perfused (4% paraformaldehyde/0.01M PBS). The brain tissue was cryoprotected (Section 2.1) and 40µm coronal sections were cut on a cryostat.

6.2.3 Immunohistochemistry and analysis

To examine the Aβ plaque load in the Tg2576 mice at the end of their treatment regime, the percentage area occupied by Aβ plaques, or 'amyloid load', was assessed in five tissue sections from each Tg2576 mouse. The five coronal sections per animal were spaced 800-1200µm apart, and the middle section of the five was situated at mid-hippocampal level. All sections were treated with formic acid (Section 2.3.1) and double immunolabelled with rabbit anti-pan-Aβ and mouse anti-α-internexin antibodies (Table 2.2). Double labelling fluorescent immunohistochemistry was performed as previously described (Section 2.3.3), except that secondary fluorescent antibodies were used at a dilution of 1:1000. Additionally, another five sections per Tg2576 mouse were

stained with thioflavine s (Section 2.2.1) and labelled for α-internexin. The Aβ plaque and thioflavine s-stained plaque loads were determined for all of the neocortex superior to the rhinal fissure, and the hippocampus (when present) in one hemisphere of each section of tissue. Specifically, low magnification images (25x) were obtained to determine the total area of cortex and hippocampus present in each tissue section, while higher magnification images (400x) were utilised to assess the total Aβ plaque area for each tissue section. All image collection and area quantitation was performed blinded to case type. Quantitative data was analysed utilising a two tailed t-test (two sample unequal variance).

To assess other possible effects of MTIIA administration sections from each Tg2576 mouse were also immunolabelled with antibodies to Aβ and α-internexin, NFM, hyperphosphorylated-tau, MAP2, GFAP or MTI/II (Table 2.2). In addition, age-matched wild type mice were double immunolabelled with rabbit anti-pan-Aβ and mouse anti-α-internexin antibodies. The presence of Aβ plaque-associated DNs, dendritic alterations, gliosis and MTI/II-labelling was investigated.

6.3 RESULTS

6.3.1 β-amyloid and thioflavine s plaque loads following MTIIA treatment of Tg2576 mice

To assess the effects of MTIIA administration on the pathology present in Tg2576 mice the Aβ and thioflavine s plaque loads were determined following 12 weeks of treatment with either MTIIA or PBS beginning at either 10 or 12 months of age. There was no significant difference in the percentage area occupied by Aβ or thioflavine s plaques in the cortex or hippocampus of Tg2576 mice treated with MTIIA compared to Tg2576 mice treated with PBS, for both treatment regimes (Figure 6.1). There was, however, a trend towards the Tg2576 mice treated with MTIIA exhibiting lower Aβ and thioflavine s plaque loads than Tg2576 mice treated with PBS (Figure 6.1A,B). In addition, no Aβ-labelling or abnormal α-internexin-labelling was present in the age-matched control wild type mice injected intraperitoneally with MTIIA or PBS.

6.3.2 β-amyloid plaque-associated neuronal pathology and MTI/II immunolabelling in Tg2576 mice following MTIIA treatment

The presence of Aβ plaque-associated neuronal pathology and MTIIA immunolabelling in Tg2576 mice were also qualitatively analysed following 12 weeks of MTIIA or PBS administration, initiated at either 10 or 12 months of age. All PBS and MTIIA treated Tg2576 mice demonstrated α-internexin- and NFM-labelled ring- and bulb-like DNs, along with Aβ plaque-associated non-classical punctate and fine-thread-like hyperphosphorylated-tau-labelling (Figure 6.2). In addition, both MTIIA and PBS treated mice exhibited MAP2-labelled dendrites that deflected around or terminated at the margins of Aβ plaques (as shown in Figure 5.2C-F). Finally, there were no robust differences in the levels of MTI/II and GFAP immunolabelling between PBS and MTIIA treated mice for both treatment regimes (Figure 6.3).

Figure 6.1

There were no significant differences between the Aβ or thioflavine s plaque loads in the hippocampus or cortex of Tg2576 mice treated with MTIIA compared to the control PBS treated mice. When the percentage area of cortex occupied by Aβ-labelled plaques (**A**) or thioflavine s-stained plaques (**B**) for each animal (black circles) treated with MTIIA from 10-13 months of age were compared to control PBS treated Tg2576 mice, the average plaque loads (black lines) for the MTIIA and PBS treatment groups were not significantly different. Similarly, when Tg2576 mice were administered either MTIIA or PBS from 12-15 months of age, the average cortical area (black lines) occupied by Aβ-labelled plaques (**C**) or thioflavine s-stained plaques (**D**) were not significantly different.

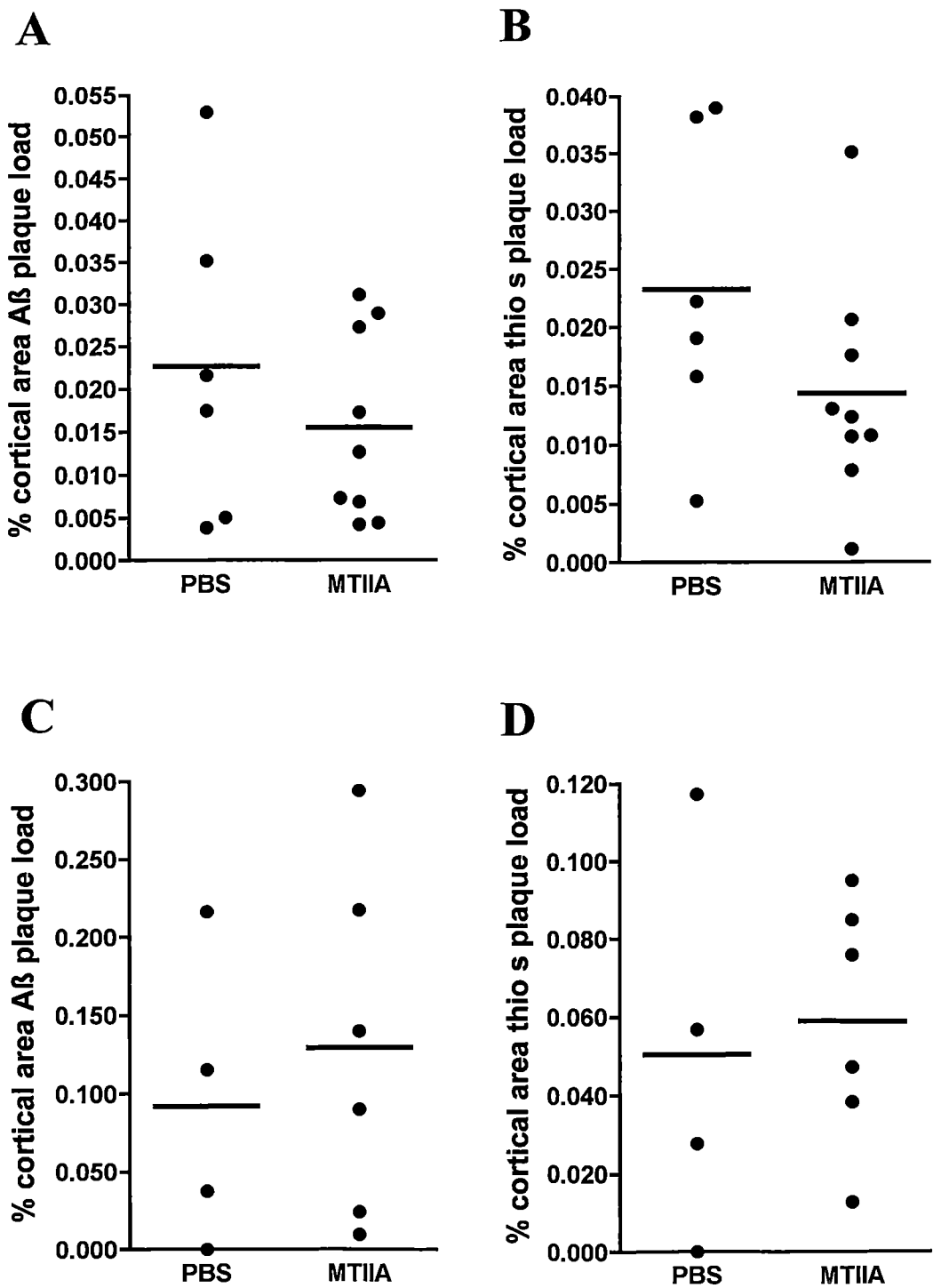

A
% cortical area Aß plaque load
0.055
0.050
0.045
0.040
0.035
0.030
0.025
0.020
0.015
0.010
0.005
0.000
PBS
MTIIA
B
% cortical area thio s plaque load
0.040
0.035
0.030
0.025
0.020
0.015
0.010
0.005
0.000
PBS
MTIIA
C
% cortical area Aß plaque load
0.300
0.250
0.200
0.150
0.100
0.050
0.000
PBS
MTIIA
D
% cortical area thio s plaque load
0.120
0.100
0.080
0.060
0.040
0.020
0.000
PBS
MTIIA

Figure 6.2

All MTIIA and PBS treated Tg2576 mice exhibited similar Aβ plaque-associated neuronal pathology. Tg2576 mice treated with either MTIIA (**A**) or PBS (**B**) from 12-15 months of age demonstrated Aβ plaque (red) associated α-internexin-labelled (green) bulb- (arrows) and ring-like (arrow heads) DNs. Similarly, both bulb- (arrows) and ring-like (arrow heads) NFM-labelled (green) DNs were associated with Aβ plaques (red) in Tg2576 mice that were administered either MTIIA (**C**) or PBS (**D**) from 12-15 months of age. Additionally, non-classical punctate hyperphosphorylated-tau-labelling (green, arrows) was also associated with Aβ plaques (red) in Tg2576 mice that were treated with either MTIIA (**E**) or PBS (**F**) from 12-15 months of age. Scale bar = 10μm.

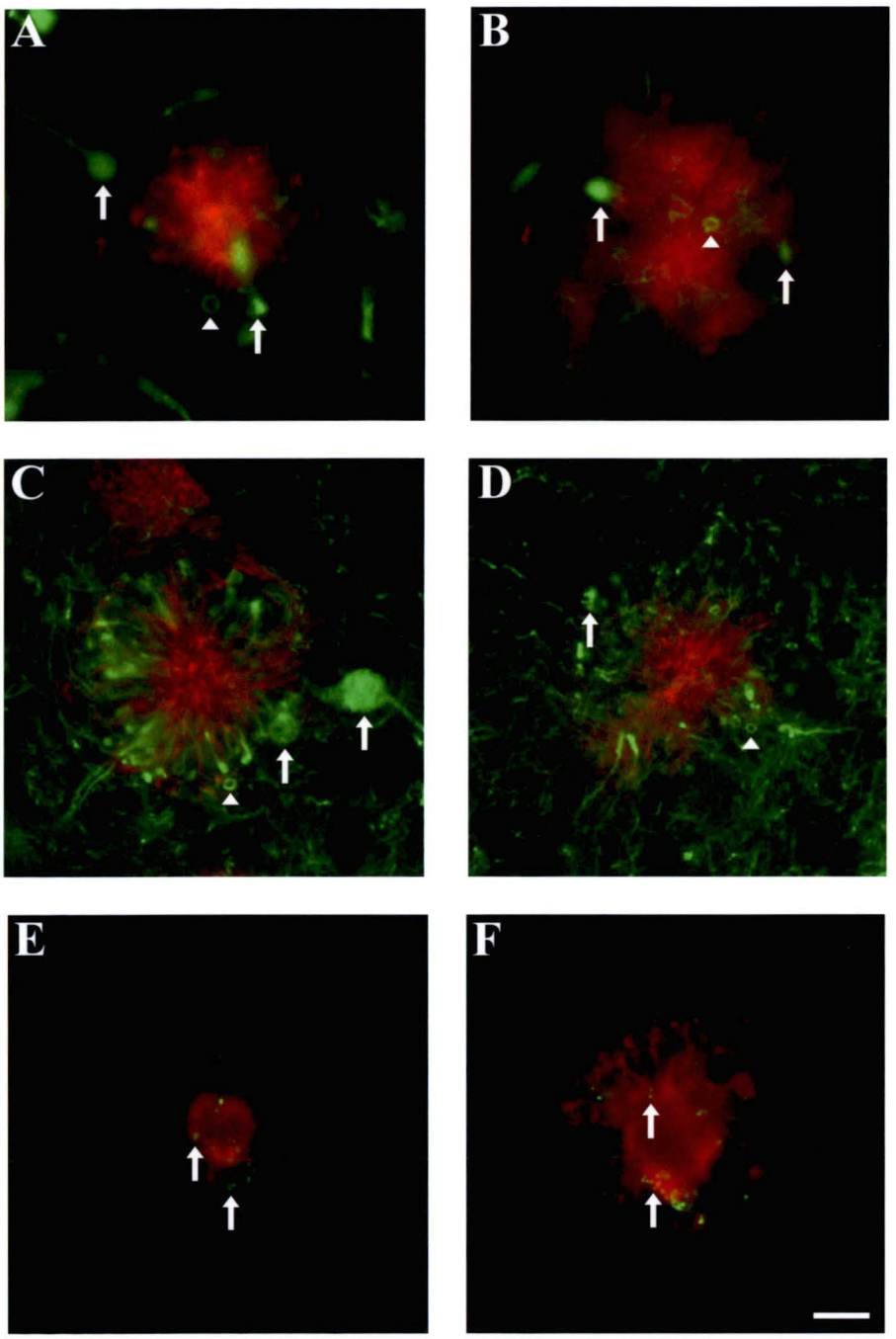
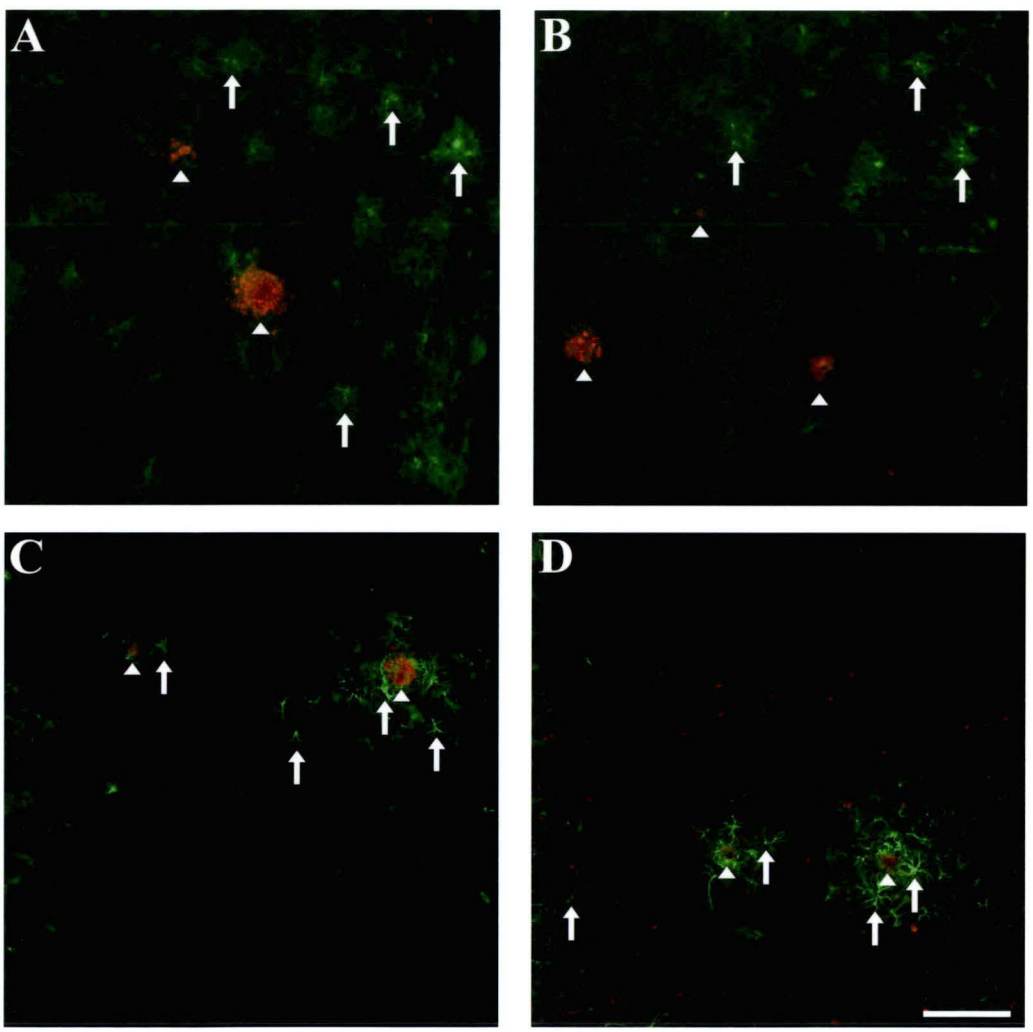

A
B
C
D
E
F

Figure 6.3

There was no robust difference in the levels of MTI/II or GFAP immunolabelling between Tg2576 mice treated with MTIIA compared to PBS treated control mice for either treatment regime. Metallothinein I/II-labelling (green) was not robustly increased or decreased when Tg2576 mice treated with PBS (**A**) from 12-15 months of age were compared to comparable MTIIA treated Tg2576 mice (**B**). Metallothionein I/II-labelled astrocytes (arrows) were not specifically associated with Aβ-labelled (red) plaques (arrow heads) in Tg2576 mice. Similarly, levels of GFAP (green) immunolabelling were comparable in Tg2576 mice that were treated with PBS (**C**) and MTIIA (**D**) from 12-15 months of age. Glial fibrillary acidic protein-labelled astrocytes (arrows) were often specifically associated with Aβ-labelled (red) plaques (arrow heads). Scale bar: **A,B** =70μm, **C,D** = 90μm.


A
B
C
D

6.4 DISCUSSION

This pilot study did not find significant changes in the Aβ or thioflavine s plaque loads of Tg2576 mice treated with MTIIA, compared to control Tg2576 mice treated with PBS. However, there was a non-significant trend towards MTIIA treated transgenic mice exhibiting lower Aβ and thioflavine s plaque loads than PBS treated Tg2576 mice. In addition, no changes in MTI/II or GFAP immunolabelling, Aβ plaque-associated DNs or dendrites were present when MTIIA treated Tg2576 mice were compared to PBS treated Tg2576 mice. Although this pilot study has not established any significant differences between the MTIIA and PBS treated Tg2576 mice, the administration of MTIIA to Tg2576 mice warrants further investigation.

Many facets of the therapeutic administration of MTIIA to Tg2576 mice may be modified for future studies, including the number of animals used, route of MTIIA administration, dose of MTIIA utilised, age at which MTIIA treatment is initiated and the length of the treatment regime. The current pilot study was undertaken with a limited number of Tg2576 animals available, so no mice were treated with zinc to control for the zinc chelated to the administered MTIIA. Had the results of this pilot study yielded statistically significant results, a zinc only Tg2576 treatment group would have been added to the data set. Ideally, an increased number of Tg2576 mice in both the MTIIA and PBS treatment groups would also give future studies more power to determine whether MTIIA is having a beneficial effect on Aβ plaques, DNs and gliosis. The range of Aβ and thioflavine s plaque loads present in the control PBS treated Tg2576 mice (Figure 6) show that the plaque load is highly variable from animal to animal in this transgenic mouse model. Therefore, it is likely that a higher number of mice need to be included in the current pilot study for any potential effects of MTIIA to be detected above the normal variability in Aβ or thioflavine s plaque load. Alternatively, it is also possible that MTIIA is not an efficient therapeutic agent for the treatment of transgenic AD mouse models and human AD. Although BBB disruption and leakage have been reported in both Tg2576 mice (Ujiie et al., 2003; Kumar-Singh et al., 2005; Dickenstein et al., 2006) and human AD (Algotsson and Winblad, 2007;

Bowman et al., 2007; Matsumoto et al., 2007; Zipser et al., 2007), it is possible that the disruption of the BBB in Tg2576 mice is not sufficient for therapeutic levels of intraperitoneally administered MTIIA to enter the CNS. Thus, future studies in Tg2576 mice may investigate the administration of MTIIA intracerebrally or increase the dose of MTIIA delivered by intraperitoneal injection. Indeed, other studies that have shown successful outcomes following intraperitoneal injection of MTII have used doses of 1-3.3 μg/g body weight daily (Giralt et al., 2002; Penkowa and Hidalgo, 2003). In addition, the time at which MTIIA administration is initiated and the length of the treatment regime could also affect the outcomes of this and future studies. The current investigation initially treated Tg2576 mice with MTIIA or PBS for 12 weeks beginning at 10 months of age. As Tg2576 mice first demonstrate Aβ plaque and Aβ plaque-associated pathology at seven months of age, which progressively increases (Hsiao et al., 1996; Kawarabayashi et al., 2001; Noda-Saita et al., 2004), and the current pilot study began a 12 week treatment course at either 10 or 12 months of age, future studies may investigate the prophylactic application of MTIIA to Tg2576 mice by beginning MTIIA treatment prior to the appearance of Aβ plaque pathology. Especially, as it is currently unknown whether MTIIA is able to aid in the disassembly and clearance of existing Aβ plaques. It is also possible that treating Tg2576 mice with MTIIA for longer periods of time would be more beneficial and result in a significant reduction in pathology.

The current study has not shown any significant difference in Aβ or thioflavine s plaque loads, or Aβ plaque-associated pathology between MTIIA and PBS treated Tg2576 mice. However, this investigation was a necessary pilot study, which provides a basis for future further investigation of the potential for MTIIA to be utilised as a therapeutic for AD.

7 DISCUSSION

Alzheimer's disease is the most common form of dementia, and currently represents a significant social and economic burden that is likely to become an increasing problem as the world's population ages (Vickers et al., 2000). The characteristic pathological hallmarks of AD include Aβ plaques, DNs, NFTs and neuropil threads, and all involve abnormal insoluble aggregates of proteins. The proteins involved in these pathological proteinaceous aggregates include Aβ, cytoskeletal neurofilaments and the microtubule associated protein tau, and all have regular physiological roles in the healthy aged brain. Thus, aberrant protein processing, folding, degradation and phosphorylation have all been implicated in the ability of Aβ, neurofilaments and tau to aggregate abnormally (as reviewed in Miller et al., 2002; Chaudhuri and Paul, 2006; Mi and Johnson, 2006; Stockley and O'Neill, 2007), but the exact event or set of events that instigates the development of AD pathology is currently unknown. Many genetic and environmental factors have been associated with AD, but age is still the greatest risk factor for the development of this disease. Although cellular biochemical pathways have been proposed to link the formation of extracellular Aβ plaques and abnormal intracellular processing of the APP with the intracellular accumulation of cytoskeletal and cytoskeletal associated proteins in NFTs and DNs (Mandelkow et al., 1992; Ferreira et al., 1997; Lau and Ahlijanian, 2003; Ryder et al., 2003; De Felice et al., 2007), the primary cause of AD and the exact mechanisms by which AD progresses and neurons degenerate and die have not yet been determined. Therefore, the central aim of this thesis was to investigate the cellular mechanisms involved in the progression of AD that lead to cellular dysfunction and death. A more complete understanding of the aetiology and progression of AD are vital for the development of more efficient therapeutics for AD as current treatments for AD only relieve symptoms, and do not slow or stop its progression.

Studying AD with the aim of identifying the primary causative agent or the specific cellular mechanisms and pathways involved in disease progression has been difficult:

the brain pathology associated with AD is complex, and postmortem human tissue provides only uncontrolled and variable time points in the dynamic process of disease progression. Thus, some key questions in the field of AD research still need to be resolved, including: 1) What is the primary causative agent of AD? 2) How does AD progress, and how are the pathological characteristics of AD related to one another? 3) What causes cells to die in AD and how?

It has been suggested that the final pathway to cell death in AD involves NFT formation, cell cycle or plasticity dysregulation, apoptosis or cell death that is neither classical necrosis or apoptosis (reviewed in Vickers et al., 2000; Jellinger and Stadelmann, 2001). As highlighted in Chapter One, the literature regarding apoptosis in AD is often confounding and contradictory. Numerous studies report both increased levels of DNA fragmentation and apoptosis-related proteins in AD brains compared to control brains (Su et al., 1994a; Dragunow et al., 1995; Satou et al., 1995; Smale et al., 1995; Cotman and Su, 1996; Lassmann, 1996; Troncoso et al., 1996; Kitamura et al., 1997; Lucassen et al., 1997; Nagy and Esiri, 1997; Su et al., 1997; Sugaya et al., 1997; Masliah et al., 1998; Sheng et al., 1998; Stadelmann et al., 1998; Giannakopolous et al., 1999; Stadelmann et al., 1999; Lu et al., 2000; Overmyer et al., 2000; Rohn et al., 2001a, Rohn et al., 2002; Blanchard et al., 2003; Pompl et al., 2003; Zhao et al., 2003a; Zhao et al., 2003b; Del Villar and Miller, 2004; Kang et al., 2005), whereas many investigations find that the levels of the same apoptosis-related proteins and DNA fragmentation are similar or decreased in AD brains compared to healthy aged brains (Lucassen et al., 1997; MacGibbon et al., 1997; Nagy and Esiri, 1997; Desjardins and Ledoux, 1998; Kitamura et al., 1998; Stadelmann et al., 1998; Engidawork et al., 2001; Raina et al., 2001; Gastard et al., 2003; Wu et al., 2005). Similar discrepancies arise when the association of apoptotic-related proteins and DNA fragmentation with Aβ plaques and NFTs is examined (Su et al., 1994a; Lassmann et al., 1995; De la Monte et al., 1997; MacGibbon et al., 1997; Su et al., 1997; Tortosa et al., 1998; Broe et al., 2001; Ferrer et al., 2001; Giannakopolous et al., 2001; Jellinger and Stadelmann, 2001; Rohn et al., 2001b; Gastard et al., 2003; Guo et al., 2004; Kobayashi et al., 2004; Wu et al., 2005). Thus, it is difficult to deduce the importance of apoptosis in the AD process from the existing

data (Roth, 2001; Raina et al., 2003). To clarify this contentious area of AD research the current study investigated the presence of apoptotic-like nuclei and the levels of a comprehensive range of apoptosis-related proteins from the intrinsic and extrinsic apoptotic pathways in control, preclinical AD and AD cases. There were no robust changes in the level of apoptotic nuclei or immunolabelling for aC3, aC8, aC9, Bax, Bcl-2, cyto c or TRADD that distinguished between case types. In addition, there were no substantial changes in the levels of Bax, Bcl-2 and TRADD mRNA extracted from the same set of cases that were immunolabelled for apoptotic-related proteins. These results challenge the proposition that apoptosis plays a major role in the progression or pathogenesis of AD, and imply that apoptosis may merely represent a terminal pathway of cell death for neurons unable to withstand the ravages of AD pathology any longer. Due to the chronic nature of AD and the short period of time it takes a cell to undergo apoptosis, the number of apoptotic cells present within AD brains at any time should be very low (Perry et al., 1998; Jellinger and Stadelmann, 2000). Therefore, even though apoptotic morphology and apoptotic-related proteins were significantly elevated in AD and preclinical AD brains compared to control brains, it is still possible that the terminal degenerative pathway of neurons in AD is apoptotic, but that the number of cells undergoing apoptosis at any one time is so low that they are not discernable above the background level of apoptosis present in the normal aged brain.

In agreement with other studies, this investigation found that apoptotic markers can be present in AD brains (Su et al., 1996b; MacGibbon et al., 1997; Nagy and Esiri, 1997; Su et al., 1997; Kitamura et al., 1998; Stadelmann et al., 1998; Tortosa et al., 1998; Giannakopolous et al., 1999; Raina et al., 2001; Rohn et al., 2001b; Rohn et al., 2002; Su et al., 2002; Gastard et al., 2003; Pompl et al., 2003; Zhao et al., 2003b; Del Villar and Miller, 2004), but still not result in extensive apoptosis. If all the cells expressing pro-apoptotic proteins completed apoptosis, a substantial neuronal loss would occur within a short period of time (Perry et al., 1998) in all case types. This suggests that apoptotic pathways are activated in these cells but do not proceed to completion. Avoidance of extensive apoptosis in AD brains (Troncoso et al., 1996; Lucassen et al., 1997; Perry et al., 1998; Stadelmann et al., 1998; Jellinger and Stadelmann, 2000; Raina

et al., 2001; Raina et al., 2003) despite the presence of apoptotic markers (Satou et al., 1995; Lucassen et al., 1997; Nagy and Esiri, 1997; Su et al., 1997; Sugaya et al., 1997 Kitamura et al., 1998; Stadelmann et al., 1998; Stadelmann et al., 1999; Rohn et al., 2001a; Rohn et al., 2002; Blanchard et al., 2003; Zhao et al., 2003b), may be due to sub-lethal activation of apoptotic pathways, perhaps caused by the excitotoxins, oxidative stress, decreased glucose metabolism and/or Aβ accumulation observed in AD and healthy aging (as reviewed in Cotman, 1998), or neurons may mount an effective defence against apoptotic cell death (Perry et al., 1998; Raina et al., 2001; Raina et al., 2003). As neurons are post-mitotic and do not readily regenerate (reviewed in Chuckowree et al., 2004; Harel and Strittmatter, 2006), it is reasonable to expect that evolution has provided some in-built defense or safe guard against widespread neuronal apoptosis. The exact mechanisms that may enable neurons to withstand apoptotic stimuli have yet to be determined.

In addition, it is also possible that discrete activation of apoptotic pathways occur. For example, in the current investigation cytoplasmic cyto c, indicating cyto c release from the mitochondria and the potential activation of the intrinsic apoptotic pathway, was present in a subset of DNs, while cyto c-labelling in the perikaryon of cortical cells was punctate. Similar release of cyto c has been reported in injured axonal segments, but was not present in the corresponding cell bodies (Büki et al., 2000). However, it should be noted that, in addition to indicating the activation of the mitochondrial apoptotic pathway, cyto c release can also signify mitochondrial damage or dysfunction. Furthermore, increasing evidence suggests that caspases may be activated for cellular processes other than apoptosis, potentially by discrete activation within cellular compartments and/or the cleavage of limited select substrates (as reviewed by Schwerk and Schulze-Osthoff, 2003).

The lack of any specific co-localisation of apoptotic-like nuclei with pathological hallmarks of AD such as NFTs or Aβ plaques in the current study and others (Troncoso et al., 1996; Lucassen et al., 1997; Sugaya et al., 1997; Broe et al., 2001) is a striking

finding. The scarcity of apoptotic-like nuclei associated with Aβ plaques does not support the proposition that Aβ plaques are directly toxic to surrounding neurons. In addition, early- and late-stage NFTs were also not co-localised with apoptotic-like nuclei or cytoplasmic cyto c immunolabelling. In contrast to NFTs in the entorhinal cortex (Vickers et al., 1992), most neocortical NFTs in AD are intracellular (Sampson et al., 1997; Vickers et al., 2003), suggesting that NFT-bearing neurons may be able to survive NFT formation for long periods of time before neurodegeneration and cell death occurs (as reviewed by Jellinger and Stadelmann, 2001). The notion that NFT formation is detrimental to cell health is being increasingly challenged, with growing evidence suggesting that tau phosphorylation and subsequent NFT formation may be by-products of protective compensatory mechanisms that protect neurons against oxidative stress (Lee et al., 2005). Specifically, neuronal loss does not directly correlate with NFT development in mouse models of AD (Andorfer et al., 2005; SantaCruz et al., 2005), while modelling of NFT formation and neuronal degeneration in the CA1 of the human hippocampus has suggested that NFT-bearing neurons survive for approximately 20 years (Morsch et al., 1999). In addition, recent research suggests that tau phosphorylation may protect neurons against apoptosis (Li et al., 2007). Cells overexpressing tau are resistant to various apoptotic stimuli, which instead induce tau hyperphosphorylation, GSK3 activation and decreases in β-catenin phosphorylation in tau expressing cells (Li et al., 2007). These data suggest an anti-apoptotic function of tau hyperphosphorylation, potentially by tau competitively inhibiting the phosphorylation, and subsequent activation, of β-catenin by GSK3 (Li et al., 2007). However, it has also recently been reported that reducing endogenous tau levels in transgenic mice expressing APP did not alter Aβ levels, but did ameliorate behavioural deficits (Roberson et al., 2007). In addition, reduced tau levels also protected transgenic and wild type mice from excitotoxicity (Roberson et al., 2007).

Examining the pathology present in human AD brain tissue has provided a wealth of information and clues as to which proteins and cellular pathways may be fundamentally involved in AD, but it is difficult to assess disease progression, the potential links

between the pathological hallmarks of AD and the cellular/molecular pathways that lead the loss of nerve cell function and cell death in the snap shots that postmortem human tissue supplies. Consequently, studying preclinical AD cases or transgenic mouse models of AD may provide important insights into the initiation, staging and maturation of AD-associated pathology (Benzing et al., 1993; Lue et al., 1996; Vickers et al., 1996; Dickson et al., 1999; Dickson and Vickers, 2001; Vickers, 2001). Preclinical AD cases are typically associated with minor cognitive deficits, and are defined as a subset of non-demented cases exhibiting widespread non-neuritic Aβ plaques (based on thioflavine s or tau-immunolabelling) in the neocortex, neurofibrillary pathology that is mostly restricted to the entorhinal formation and hippocampus, and no overt neuronal degeneration or loss (Morris et al., 1996; Price and Morris, 1999; Vickers et al., 2000). To date the study of preclinical AD cases has provided valuable information regarding AD initiation and progression. Preclinical AD cases demonstrate Aβ plaques and DNs with morphological and biochemical characteristics that differ from those in AD cases, but no tauopathy, which includes NFTs, neuropil threads and tau-labelled DNs (Morris et al., 1996; Price and Morris, 1998; Vickers et al., 2000). This suggests that Aβ plaques and DNs containing neurofilaments and APP, but not tau, are some of the earliest pathology to develop in human AD, and provides support for the Aβ cascade hypothesis. Transgenic mouse models of AD represent additional tools for researchers examining AD progression, as a range of pre- and post-pathology time points can be assessed with the added facet that the disease process can be actively and readily, interfered with. Although transgenic mouse models of AD have been extremely useful, aspects of the way in which transgenic AD mouse models fit into or compare to the schema of disease staging that occurs in human AD have been under appreciated.

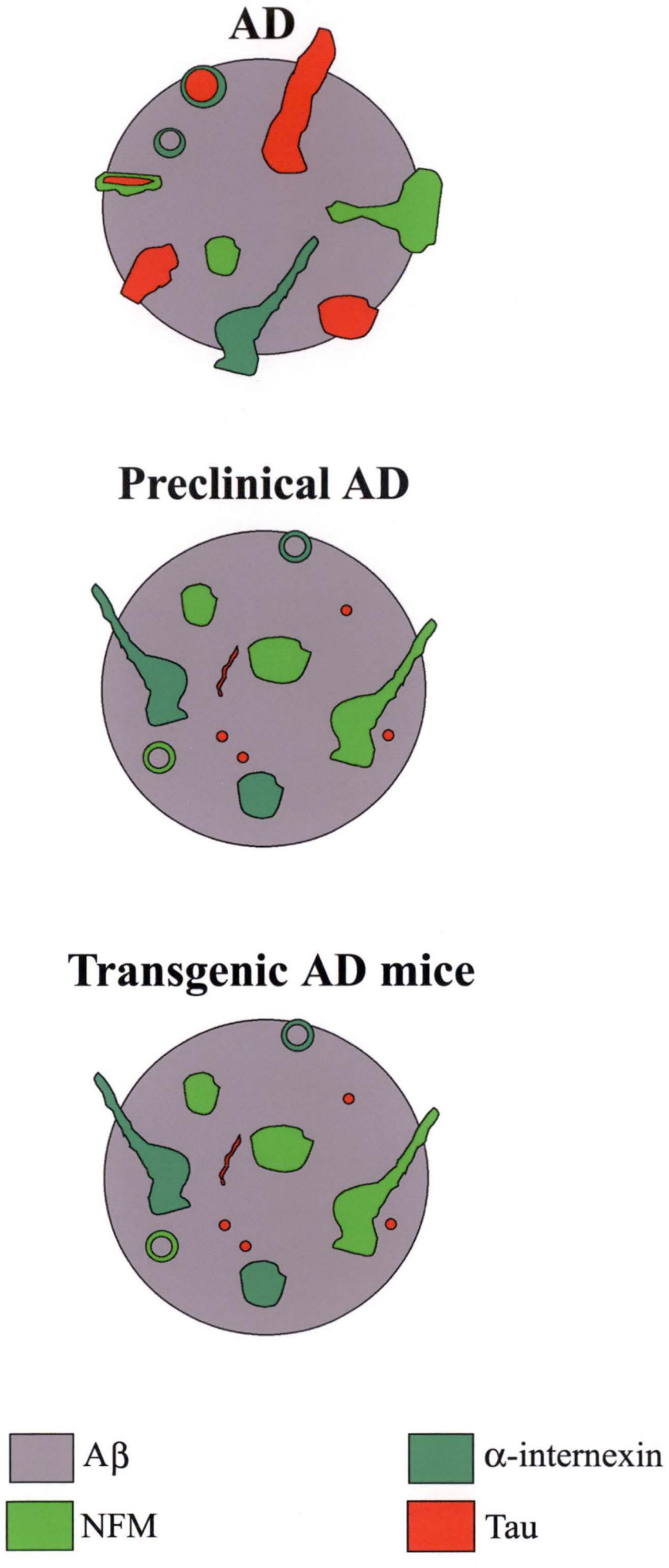
Transgenic mice overexpressing APP harbouring familial AD mutations reliably produce Aβ plaques, Aβ plaque-associated gliosis and DNs, but do not develop NFTs and neuropil threads or exhibit the dramatic neuronal loss equivalent to that in human AD cases (Games et al., 1995; Hsiao et al., 1996; Borchelt et al., 1997; Sturchler-Pierrat et al., 1997; Holcomb et al., 1998; Moechars et al., 1999; Janus et al., 2000; Mucke et al., 2000; Chishti et al., 2001; Blanchard et al., 2003; Higgins and Jacobsen, 2003;

Richards et al., 2003; Cheng et al., 2004; Kawasumi et al., 2004; Oakley et al., 2006). Overt neurodegeneration and neuron loss has only been unequivocally demonstrated in APP23 mice (Calhoun et al., 1998; Higgins and Jacobsen, 2003). The similarities between the pathology exhibited in APP transgenic AD mice and that of preclinical AD cases, namely Aβ plaques, DNs and gliosis, but no overt neurofibrillary pathology and neuron loss, suggests that transgenic AD mice may more closely model the early preclinical stages of human AD. However, no thorough analysis of Aβ plaque-associated neuronal pathology in APP transgenic AD mice had been conducted. The current investigation addressed this deficit by analysing DNs in two widely utilised APP transgenic AD mouse models, TgCRND8 and Tg2576 mice. This study determined that the biochemical and morphological characteristics of DNs in the TgCRND8 and Tg2576 mice were strikingly similar to that in human preclinical AD cases, but not AD cases. Specifically, both lines of transgenic AD mice and human preclinical AD cases demonstrated abundant NF triplet protein- and α-internexin-immunolabelled bulb- and ring-like DNs and Aβ plaque-associated punctate and fine thread-like hyperphosphorylated-tau labelling, whereas human AD cases exhibited numerous classical hyperphosphorylated-tau-labelled DNs, α-internexin-labelled bulb- and ring-like DNs and NF triplet protein-labelled bulb-like DNs (as depicted in Figure 7.1). Importantly, quantitation demonstrated that the Aβ plaques in TgCRND8 mice were highly axonopathic, as the percentage of Aβ-labelled or thioflavine s-stained plaques in TgCRND8 mice associated with DNs was equivalent to or in excess of that in human AD cases. These results indicate that the Aβ plaque-associated neuronal pathology in these mice more accurately models the early or preclinical brain changes that occur in human AD, representing a valuable model for understanding and developing treatments for preclinical AD.

With respect to the different transgenic AD mice it is important to note that the overexpression of APP harbouring familial AD mutations has resulted in the presence of Aβ plaques, DNs, astrocytosis, microgliosis and no neurofibrillary pathology on a variety of different genetic backgrounds (Games et al., 1995; Hsiao et al., 1996; Sturchler-Pierrat et al., 1997; Moechars et al., 1999; Mucke et al., 2000; Chishti et al.,

Figure 7.1

The morphological and neurochemical characteristics of Aβ plaques associated with DNs in TgCRND8 and Tg2576 mice are identical to those in human preclinical AD, but not AD cases. The transgenic AD mice, preclinical AD and AD cases exhibit NF- and α-internexin-labelled bulb-like DNs, and α-internexin-labelled ring-like DNs. NF-labelled ring-like DNs are abundant in TgCRND8 and Tg2576 mice and preclinical AD cases, but rare in AD cases. While AD cases demonstrate numerous classical angular elongated tau-labelled DNs, which are rarely observed in the transgenic AD mice and preclinical AD cases. However, preclinical AD cases and TgCRND8 and Tg2576 mice do exhibit punctate and fine-thread like tau-labelling associated with Aβ plaques. In addition, a subset of NF- and α-internexin-labelled DNs with a core of tau immunolabelling are observed in AD cases.


AD
Preclinical AD
Transgenic AD mice
Aβ
α-internexin
NFM
Tau

2001; Higgins and Jacobsen, 2003). The development of such AD-like pathology in transgenic AD mice with different genetic backgrounds provides strong evidence that the overexpression of mutant APP results in Aβ deposition and the subsequent development of Aβ plaque associated DNs and gliosis. Thus, investigating DN phenotype in two mouse models of AD that overexpress different APP mutants of different genetic backgrounds also indicates that the preclinical AD DN phenotype exhibited by Tg2576 and TgCRND8 mice is robust and likely applies to other transgenic AD mouse models. Ideally future studies may investigate the DN phenotype and other AD pathology present in not only Tg2576 and TgCRND8 mice, which represent relatively slow and fast pathology acquisition, but also in PSAPP, TAPP and 3xTg-AD mice to determine whether the expression of mutant PS1 and/or tau alters DN characteristics and the development of AD-like pathology over time. Finally, with regards to utilising transgenic AD mice as platforms to test potential pharmacotherapeutics, transgenic AD mice that overexpress mutant APP and PS1 that rapidly develop AD pathology, such as the TgCRND8 and probably PSAPP mice, would be effective for testing therapeutics to be administered to the preclinical or early stages of human AD, before overt cell loss and neurofibrillary pathology has developed. Although, therapeutics for the treatment of end stage human AD should be tested in transgenic AD mice with established neurofibrillary pathology such as the TAPP or 3xTg-AD mice.

Having characterised the neuronal pathology of two widely used transgenic AD mouse models, the final aim of this thesis was to investigate the potential of MTIIA as a therapeutic intervention for AD. Although the results of this study were not statistically significant, there was a trend towards lower Aβ-labelled and thioflavine s-stained plaque loads in the MTIIA treated Tg2576 mice compared to the PBS treated control mice. Tg2576 mice are currently being bred and aged to further investigate alternate MTIIA treatment regimes including different doses, initiation time points and length of the treatment and differing routes of administration, all with higher numbers of animals in the treatment groups.

With respect to the cellular mechanisms of disease progression, it is intriguing that despite exhibiting Aβ plaques and DNs, transgenic APP AD mice do not develop the full spectrum of AD pathology, as the Aβ cascade hypothesis would predict. Why PHFs, NFTs, neuropil threads and classical tau-labelled DNs do not develop in transgenic APP AD mice is currently unknown (McGowan et al., 2006). Mouse tau is almost homologous to human tau and is able to form filaments *in vitro* (Kampers et al., 1999), and NFTs are produced *in vivo* in mice when human mutant tau harbouring mutations associated with hereditary frontotemporal dementia and parkinsonism, are overexpressed (Lewis et al., 2000; Gotz et al., 2001; Zhang et al., 2004a; Terwel et al., 2005). However, mice overexpressing all six human tau isoforms but not endogenous mouse tau develop PHFs and pathological accumulations of PHF-tau in the soma and dendrites of neurons (Andorfer et al., 2003), but when the entire human wild type tau gene is overexpressed in mice with endogenous tau expression, no obvious histopathological phenotype develops (Duff et al., 2000), suggesting that endogenous mouse tau is inhibitory to the formation of tau filaments (Gotz et al., 2004). It is also possible that other factors or cellular pathways required for the development of tauopathy are missing or are limited in the mouse brain (Phinney et al., 2003). Furthermore, the lack of extensive neuronal loss in transgenic APP AD mice may be due to the lack of neurofibrillary pathology, if this pathology specifically causes neurons to degenerate and die. Alternatively, perhaps the relatively fast development of Aβ plaques in transgenic AD mouse models and the short life span of mice does not allow enough time for Aβ plaques to cause chronic stress and damage that may result in neuronal loss. In this respect, it has been shown that the Aβ in human Aβ plaques is chemically modified, including N-terminal truncation, crosslinking and isomerisation, whereas equivalent Aβ modifications are not present in transgenic AD mice (Kuo et al., 2001). The deposition of various other proteins, cations and lipids in Aβ plaques that occurs in human AD may also differ in transgenic AD mouse models (Maynard et al., 2002). Although the biological significance of co-deposited molecules and the chemical modification of Aβ within Aβ plaques are unclear (Phinney et al., 2003), it may influence the toxicity of Aβ plaques (Higgins and Jacobsen, 2003). In addition, the development of different Aβ plaque types in transgenic AD mouse models does not precisely mimic the progression

of Aβ plaque types exhibited in human AD. For example, 70% of Aβ plaques in 18-month old Tg2576 mice were diffuse (Tomidokoro et al., 2001; Sasaki et al., 2002), while in APP23 mice more than 90% of Aβ plaques were dense-cored and diffuse Aβ plaques were predominantly exhibited in mice with high Aβ plaque loads (Calhoun et al., 1998). Instead, human AD cases exhibit a higher relative proportion of fibrillar and dense-core Aβ plaques than preclinical AD cases (Dickson and Vickers, 2001).

A variant of the amyloid cascade hypothesis has been proposed, in which Aβ plaque formation causes compression of the neuropil and sufficient structural injury to initiate axonal cytoskeletal changes, resulting in DN formation, and potentially the subsequent development of neurofibrillary pathology, neurodegeneration and cell death over the chronic time course of this disease (as previously reviewed in Vickers, 1997; Vickers et al., 2000). In support of the proposition that Aβ plaque formation structurally displaces the neuropil, fewer dendrites, axons and neuronal cell bodies are located within the area occupied by Aβ plaques in human AD and transgenic AD mouse models (DeWitt and Silver, 1996; Knowles et al., 1998; Le et al., 2001; Tomikodoro et al., 2001; Adlard and Vickers, 2002; Moolman et al., 2004; Tsai et al., 2004). In addition, the NF triplet protein and α-internexin-labelled ring- and bulb-like DNs observed following *in vitro* and *in vivo* axonal injury are strikingly similar to those seen in AD brains and, as described in this study, transgenic AD mice (Masliah et al., 1993; Meller et al., 1994; DeWitt and Silver, 1996; Masliah et al., 1996b; Su et al., 1996a; Vickers et al., 1996; Christman et al., 1997; Irizarry et al., 1997; King et al., 1997; Nakamura et al., 1997; Dickson et al., 2000; Chishti et al., 2001; King et al., 2001; Le et al., 2001; Chuckowree and Vickers, 2003; Bussière et al., 2004; Dickson et al., 2005). Furthermore, dephosphorylated NF triplet proteins are abnormally present in axons following physical trauma and within the axonopathic changes in human AD and transgenic AD mice (Masliah et al., 1993; Meller et al., 1994; DeWitt and Silver, 1996; Su et al., 1996a; Vickers et al., 1996; Christman et al., 1997; King et al., 1997; Nakamura et al., 1997; Dickson et al., 2000; King et al., 2001; Le et al., 2001; Chuckowree and Vickers, 2003; Dickson et al., 2005). Apoptotic-related proteins including activated caspases and cytoplasmic cyto c were evident in DNs in transgenic AD mice and in human AD in the

current investigation and others (De la Monte et al., 1997; MacGibbon et al., 1997; Nagy and Esiri et al., 1997; Tortosa et al., 1998; Giannakopolous et al., 2001; Rohn et al., 2002; Su et al., 2002; Blanchard et al., 2003; Su et al., 2003; Guo et al., 2004; Wu et al., 2005; Albrecht et al., 2007), and are also present in axons following axonal injury or disruption (Springer et al., 1999; Büki et al., 2000; Wingrave et al., 2003; DeRidder et al., 2006). This expanding body of evidence supports the mass effect variant of the amyloid cascade hypothesis and strongly suggests that Aβ plaque induced neuronal injury may play an important role in the initiation of DN pathology in AD and in AD progression.

This thesis has substantially contributed to our growing understanding of the staging of the pathological changes that underlie AD, clarifying the role of apoptosis in AD and strongly suggesting that transgenic APP AD mouse models accurately mimic the Aβ plaque-associated neuronal pathology of human preclinical AD. Furthermore, the reported pilot study of the effect of MTIIA will lead to further investigation of this potential therapeutic approach in the future.

7.1 CONCLUSIONS

- ***There is no difference in the levels of apoptosis-related proteins and apoptotic nuclear morphology between preclinical AD, AD and control cases, suggesting that apoptosis does not play a major role in the progression of AD or the pathogenesis of the pathological hallmarks of AD or neuronal degeneration.***

- ***Activation of apoptotic pathways can occur without extensive terminal apoptosis within the brain.***

- ***As indicated by the lack of apoptotic nuclei and the presence of punctate cyto c-labelling in the soma of NFT-bearing neurons, NFTs may not be as severely detrimental to neuronal health as previously suggested.***

- ***Cytochrome c may be a transient and early marker in Aβ plaque-associated DNs, and its presence in the cytoplasm of DNs may indicate activation of apoptotic pathways and/or mitochondrial dysfunction.***

- ***Neurofilament triplet protein and α-internexin containing DNs precede the appearance of hyperphosphorylated-tau-labelled DNs, suggesting that DNs may mature from NF-labelled DNs to tau-labelled DNs as AD progresses.***

- ***The neurochemistry and morphology of DNs in two widely used transgenic mouse models of AD recapitulates the DN pathology present in preclinical AD cases, but not AD cases, indicating that transgenic APP AD mouse models more accurately model the preclinical stages of AD and early Aβ plaque-associated neuronal pathology.***

7.2 FUTURE DIRECTIONS

- ***Future studies could further investigate both the DN phenotype and other AD pathology present in PSAPP, TAPP and 3xTg-AD mice to determine whether the expression of mutant PS1 and/or tau alters DN characteristics and the development of AD-like pathology over time.***

- ***It would also be of interest to build upon the data regarding apoptosis in AD, by investigating apoptosis in transgenic mouse models of AD including Tg2576, PSAPP and 3xTg-AD mice, with respect to apoptotic nuclear morphology and the levels of apoptotic-related proteins to compare to that present in human AD tissue.***

- ***To further increase our understanding of disease progression in AD, a set of hereditary AD cases expressing PS1 harbouring familial mutations that exhibit accelerated AD progression could be examined for DN phenotype and the load***

and distribution pattern of Aβ plaques and NFTs in comparison to a cohort of sporadic preclinical AD and AD cases.

- ***As discussed in the current study, select subsets of neurons, notably those that express NF triplet proteins, are selectively vulnerable to degeneration in AD. To investigate the cellular basis of this select neuronal vulnerability, NFL knock out mice could be crossed with transgenic APP/PS1 mice to ascertain the importance of the NF triplet proteins to the local response of neurites to axonal injury in vitro, and Aβ plaque induced damage in vivo.***

- ***The current investigation, along with numerous other studies, highlights the damaging effects of Aβ plaques on the surrounding neuropil, but exactly how the earliest disruption of the cortical network by Aβ plaque formation correlates with cognitive deficits remains unknown. Thus, yellow fluorescent protein transgenic mice could be crossed with APP/PS1 mice and the response of fluorescent neurites to Aβ plaques could be assessed in fixed tissue and by two-photon scanning laser microscopy in conjunction with behavioural testing at several time points.***

- ***Although the current pilot study did not result in any significant differences between MTIIA and PBS treated Tg2576 mice, the use of MTIIA as a potential prophylactic therapeutic agent could still be investigated. Future studies could utilise APP/PS1 transgenic AD mice, involve a higher number of animals per treatment group, and administer a higher dose of MTIIA from six to twelve months of age.***

8 REFERENCES

Adams JM, Cory S (2001) Life-or-death decisions by the Bcl-2 protein family. Trends in Biochem Sci 26:61-66.

Adlard PA, West AK, Vickers JC (1998) Increased density of metallothionein I/II-immunopositive cortical glial cells in the early stages of Alzheimer's disease. Neurobiol Dis 5:349-356.

Adlard PA, Vickers JC (2002) Morphologically distinct plaque types differentially affect dendritic structure and organisation in the early and late stages of Alzheimer's disease. Acta Neuropathol 103:377-383.

Adlard PA, Bush AI (2006) Metals and Alzheimer's disease. J Alzheimer's Dis 10:145-163.

Adlard PA, Barnham KJ, Cappai R, Gautier E, Kok GB, Masters CL, Cherny RA, Bush AI (2007) Treatment of Alzheimer transgenic mice with the second-generation 8-OH quinoline, PBT2, ameliorates Aβ burden and improves cognitive performance within days. International Brain Research Organisation World Congress of Neuroscience: Melbourne, Australia.

Albrecht S, Bourdeau M, Bennett D, Mufson EJ, Bhattacharjee M, LeBlanc AC (2007) Activation of caspase-6 in aging and mild cognitive impairment. Amer J Pathol 170:1200-1209.

Algotsson A, Winblad B (2007) The integrity of the blood-brain barrier in Alzheimer's disease. Acta Neurol Scand 115:403-408.

Ali SM, Siedlak SL, Gonzalez-DeWhitt PA, Altman RA, Glendening JM, Lowery DE, Savage MJ, Polites HG, Perry G, Greenberg BD (1996) Artifactual strain-specific signs of incipient brain amyloidosis in APP transgenic mice. Neurobiol Aging 17:223-234.

Alvarez XA, Cacebelos R, Laredo M, Couceiro V, Sampedro C, Varela M, Corzo L, Fernandez-Novoa L, Vargas M, Aleixandre M, Linares C, Granizo E, Muresanu D, Moessler H (2006) A 24-week, double-blind, placebo-controlled study of three dosages of Cerebrolysin in patients with mild to moderate Alzheimer's disease. Eur J Neurol 13:43-54.

Andorfer C, Kress Y, Espinoza M, de Silva R, Tucker KL, Barde YA, Duff K, Davies P (2003) Hyperphosphorylation and aggregation of tau in mice expressing normal human tau isoforms. J Neurochem 86:582-590.

Andorfer C, Acker CM, Kress Y, Hof PR, Duff K, Davies P (2005) Cell-cycle re-entry and cell death in transgenic mice expressing nonmutant human tau isoforms. J Neurosci 25:5446-5454.

Arbel M, Yacoby I, Solomon B (2005) Inhibition of amyloid precursor protein processing by β-secretase through site-directed antibodies. Proc Natl Acad Sci U S A 102:7718-7723.

Arendash GW, Garcia MF, Costa DA, Cracchiolo JR, Wefes IM, Potter H (2004) Environmental enrichment improves cognition in aged Alzheimer's transgenic mice despite stable β-amyloid deposition. NeuroReport 15:1751-1754.

Arendt T (2001) Disturbance of neuronal plasticity is a critical pathogenetic event in Alzheimer's disease. Int J Devl Neurosci 19:231-245.

Arendt T (2003a) Synaptic plasticity and cell cycle activation in neurons are alternative effector pathways: the 'Dr. Jekyll and Mr. Hyde concept' of Alzheimer's disease or the yin and yang of neuroplasticity. Prog Neurobiol 71:83-248.

Arendt T, Stieler J, Strijdstra AM, Hut RA, Rudiger J, Van der Zee EA, Harkan T, Holzer M, Hartig W (2003b) Reversible paired helical filament-like phosphorylation of tau is an adaptive process associated with neuronal plasticity in hibernating animals. J Neurosci 23:6972-6981.

Armstrong RA (1998) β-amyloid plaques: stages in life history or independent origin? Dement Geriatr Cogn Disord 9:227-238.

Arnold SE, Hyman BT, Flory J, Damasio AR, Van Hoesen GW (1991) The topographical and neuroanatomical distribution of neurofibrillary tangles and neuritic plaques in the cerebral cortex of patients with Alzheimer's disease. Cereb Cortex 1:103-116.

Arriagada PV, Growdon JH, Hedley-Whyte ET, Hyman BT (1992) Neurofibrillary tangles but not senile plaques parallel duration and severity of Alzheimer's disease. Neurology 42:631-639.

Arvanitakis Z, Wilson RS, Bienias JL, Evans DA, Bennett DA (2004) Diabetes mellitus and risk of Alzheimer's disease and decline in cognitive function. Arch Neurol 61:661-666.

Asai M, Hattori H, Iwata N, Saido TC, Sasagawa N, Szabo B, Hashimoto Y, Maruyama K, Tanuma S-I, Kiso Y, Ishuira S (2006) The novel β-secretase inhibitor KMI-429 reduces amyloid β peptide production in amyloid precursor protein transgenic and wild-type mice. J Neurochem 96:533-540.

Aschner M, Cherian G, Klaassen C, Palmiter R, Erickson J, Bush A (1997) Metallothioneins in the brain - the role in physiology and pathology. Toxicol Appl Pharmacol 142:229-242.

Atwood CS, Moir RD, Huang X, Scarpa RC, Bacarra NM, Romano DM, Hartshorn MA, Tanzi RE, Bush AI (1998) Dramatic aggregation of Alzheimer Abeta by Cu(II) is induced by conditions representing physiological acidosis. J Biol Chem 273:12817-12826.

Bacskai BJ, Kajdasz ST, McLellan ME, Games D, Seubert P, Schenk D, Hyman BT (2002) Non-Fc-mediated mechanisms are involved in clearance of amyloid-β in vivo by immunotherapy. J Neurosci 22:7873-7878.

Bard F, Cannon C, Barbour R, Burke R-L, Games D, Grajeda H, Guido T, Hu K, Huang J, Johnson-Wood K, Khan K, Kholodenko D, Lee M, Lieberburg I, Motter R, Nguyen M, Soriano F, Vasquez N, Weiss K, Welch B, Seubert P, Schenk D, Yednock T (2000) Peripherally administered antibodies against amyloid β-peptide enter the central nervous system and reduce pathology in a mouse model of Alzheimer's disease. Nature 6:916-919.

Bard F, Barbour R, Cannon C, Carretto R, Fox M, Games D, Guido T, Hoenow K, Hu K, Johnson-Wood K, Khan K, Kholodenko D, Lee C, Lee M, Motter R, Nguyen M, Reed A, Schenk D, Tang P, Vasquez N, Seubert P, Yednock T (2003) Epitope and isotype specificities of antibodies to β-amyloid peptide for protection against Alzheimer's disease-like neuropathology. Proc Natl Acad Sci U S A 100:2023-2028.

Barghorn S, Nimmrich V, Streibinger A, Krantz C, Keller P, Janson B, Bahr M, Schmidt M, Bitner RS, Harlan J, Barlow E, Ebert U, Hillen H (2005) Globular amyloid β-

peptide1-42 oligomer - a homogenous and stable neuropathological protein in Alzheimer's disease. J Neurochem 95:834-847.

Beauchet O (2006) Testosterone and cognitive function: current clinical evidence of a relationship. Europ J Endo 155:773-781.

Bellucci A, Rosi MC, Grossi C, Fiorentini A, Luccarini I, Casamenti F (2007) Abnormal processing of tau in the brain of aged TgCRND8 mice. Neurobiol Dis 3:328-338.

Benjelloun N, Joly L-M, Palmier B, Plotkine M, Charriaut-Marlangue C (2003) Apoptotic mitochondrial pathway in neurones and astrocytes after neonatal hypoxia-ischaemia in the rat brain. Neuropath App Neurobiol 29:350-360.

Benzing WW, Ikonomovic MD, Brady DR, Mufson EJ, Armstrong DM (1993) Evidence that transmitter-containing dystrophic neurites precede paired helical filament and Alz50 formation within senile plaques in the amygdala of nondemented elderly and patients with Alzheimer's disease. J Comp Neurol 334:176-191.

Benzing WC, Mufson EJ (1995) Apolipoprotein E immunoreactivity within neurofibrillary tangles: relationship to tau and PHF in Alzheimer's disease. Exp Neurol 132:162-171.

Bergamaschini L, Rossi E, Storini C, Pizzimenti S, Distaso M, Perego C, Luigi A, Vergani C, De Simoni MG (2004) Peripheral treatment with enoxaparin, a low molecular weight heparin, reduces plaques and beta-amyloid accumulation in a mouse model of Alzheimer's disease. J Neurosci 24:4181-4186.

Berger Z, Roder H, Hanna A, Carlson A, Rangachari V, Yue M, Wszoleck Z, Ashe K, Knight J, Dickson D, Andorfer C, Rosenberry TL, Lewis J, Hutton M, Janus C (2007) Accumulation of pathological tau species and memory loss in a conditional model of tauopathy. J Neurosci 27:3650-3662.

Berkelaar M, Clarke DB, Wang Y-C, Bray GM, Aguayo AJ (1994) Axotomy results in delayed death and apoptosis of retinal ganglion cells in adult rats. J Neurosci 14:4360-4374.

Best JD, Smith DW, Reilly MA, O'Donnell R, Lewis HD, Ellis S, Wilkie N, Rosahl TW, Laroque PA, Boussiquet-Leroux C, Churcher I, Atack JR, Harrison T, Shearman MS (2007) The novel gamma secretase inhibitor N-[cis-4-[(4-chlorophenyl)sulfonyl]-4-(2,5-difluorophenyl)cyclohexyl]-1,1,1-trifluoromethanesulfonamide (MRK-560)

reduces amyloid plaque deposition without evidence of notch-related pathology in the Tg2576 mouse. J Pharmacol Exp Ther 320:552-558.

Beyreuther K, Masters CL (1997) Alzheimer's disease. The ins and outs of amyloid-beta. Nature 389:677-678.

Bierer LM, Hof PR, Purohit DP, Carlin L, Schmeidler J, Davis KL, Perl DP (1995) Neocortical neurofibrillary tangles correlate with dementia severity in Alzheimer's disease. Arch Neurol 52:81-88.

Billbault P, Lavauz T, Lahlou A, Uring-Lambert B, Gaub M-P, Ratomponirina C, Meyer N, Oudet P, Schneider F (2004) Transient Bcl-2 gene down-expression in circulating mononuclear cells of severe sepsis patients who died despite appropriate intensive care. Intensive Care Med 30:408-415.

Billings LM, Oddo S, Green KN, McGaugh JL, LaFerla FM (2005) Intraneuronal Aβ causes the onset of early Alzheimer's disease-related cognitive deficits in transgenic mice. Neuron 45:675-688.

Biswas SC, Shi Y, Vonsattel J-PG, Leung CL, Troy CM, Greene LA (2007) Bim is elevated in Alzheimer's disease neurons is required for β-amyloid-induced neuronal apoptosis. J Neurosci 27:893-900.

Blanchard V, Moussaoui S, Czech C, Touchet N, Bonici B, Planche M, Canton T, Jedidi I, Gohin M, Wirths O, Bayer TA, Langui D, Duyckaerts C, Tremp G, Pradier L (2003) Time sequence of maturation of dystrophic neurites associated with Aβ deposits in APP/PS1 transgenic mice. Exp Neurol 184:247-263.

Blanchard BJ, Chen A, Rozeboom LM, Stafford KA, Weigele P, Ingram VM (2004) Efficient reversal of Alzheimer's disease fibril formation and elimination of neurotoxicity by a small molecule. Proc Natl Acad Sci U S A 101:14326-14332.

Bloom GS, Ren K, Glabe CG (2005) Cultured cell and transgenic mouse models for tau pathology linked to β-amyloid. Biochim Biophys Acta 1739:116-124.

Bombois S, Maurage C-A, Gompel M, Deramecourt V, Mackowiak-Cordoliani M-A, Black RS, Lavielle R, Delacourte A, Pasquier F (2007) Absence of β-amyloid deposits after immunization in Alzheimer's disease with Lewy Body dementia. Ann Neurol 64:583-587.

Bondolfi L, Calhoun M, Ermini F, Kuhn HG, Wiederhold K-H, Walker L, Staufenbiel M, Jucker M (2002) Amyloid-associated neuron loss and gliogenesis in the neocortex of amyloid precursor protein transgenic mice. J Neurosci 22:515-522.

Borchelt DR, Ratovitski T, van Lare J, Lee MK, Gonzales V, Jenkins NA, Copeland NG, Price DL, Sisodia SS (1997) Accelerated amyloid deposition in the brains of transgenic mice coexpressing mutant presenilin 1 and amyloid precursor proteins. Neuron 19:939-945.

Bornemann KD, Wiederhold K-H, Pauli C, Ermini F, Stalder M, Schnell L, Sommer B, Jucker M, Staufenbiel M (2001) Aβ-induced inflammatory processes in microglia cells of APP34 transgenic mice. Amer J Pathol 158:63-73.

Borroni B, Premi E, Di Luca M, Padovani A (2007) Combined biomarkers for early Alzheimer disease diagnosis. Curr Med Chem 14:1171-1178.

Bossy-Wetzel E, Schwarzenbacher R, Lipton SA (2004) Molecular pathways to neurodegeneration. Nat Rev Neurosci 10:S1-S9.

Boutajangout A, Authelet M, Blanchard V, Touchet N, Tremp G, Pradier L, Brion J-P (2004) Characterisation of cytoskeletal abnormalities in mice transgenic for wild-type human tau and familial Alzheimer's disease mutants of APP and presenilin-1. Neurobiol Dis 15:47-60.

Bowers WJ, Mastrangelo MA, Stanley HA, Casey AE, Milo Jr LJ, Federoff HJ (2005) HSV amplicon-mediated Aβ vaccination in Tg2576 mice: differential antigen-specific immune responses. Neurobiol Aging 26:393-407.

Bowman GL, Kaye JA, Moore M, Waichunas D, Carlson NE, Quinn JF (2007) Blood-brain barrier impairment in Alzheimer disease: stability and functional significance. Neurology 68:1809-1814.

Braak H, Braak E (1988) Neuropil threads occur in dendrites of tangle-bearing nerve cells. Neuropath App Neurobiol 14:39-44.

Braak H, Braak E (1991) Neuropathological stageing of Alzheimer-related changes. Acta Neuropathol 82:239-259.

Braak E, Braak H, Mandelkow EM (1994) A sequence of cytoskeletal changes related to the formation of neurofibrillary tangles and neuropil threads. Acta Neuropathol 87:554-567.

Braak H, Braak E, Bohl J, Reintjes R (1996) Age, neurofibrillary changes, A beta-amyloid and the onset of Alzheimer's disease. Neuroscience Letters 210:87-90.

Braak H, Braak E (1997) Neuropathological assessments staging of Alzheimer-related cortical destruction. Int Psychogeriat 9:257-261.

Bratton SB, MacFarlane M, Cain K, Cohen GM (2000) Protein complexes activate distinct caspase cascades in death receptor and stress-induced apoptosis. Exp Cell Res 256:27-33.

Brendza RP, O-Brien C, Simmons K, McKeel DW, Bales KR, Paul SM, Olney JW, Sanes JR, Holtzman DM (2003) PDAPP/YFP double transgenic mice: a tool to study amyloid-β associated changes in axonal, dendritic and synaptic structures. J Comp Neurol 456:375-383.

Brendza RP, Bacskai BJ, Cirrito JR, Simmons KA, Skoch JM, Klunk WE, Mathis CA, Bales KR, Paul SM, Hyman BT, Holtzman DM (2005) Anti-Aβ antibody treatment promotes the rapid recovery of amyloid-associated neuritic dystrophy in PDAPP transgenic mice. J Clin Invest 115:428-433.

Bretch WJ, Harris FM, Chang S, Tesseur I, Yu G-Q, Xu Q, Fish JD, Wyss-Coray T, Buttini M, Mucke L, Mahley RW, Huang Y (2004) Neuron-specific apolipoprotein E4 proteolysis is associated with increased tau phosphorylation in brains of transgenic mice. J Neurosci 24:2527-2534.

Brion JP (1996) The neurobiology of Alzheimer's disease. Acta Clinica Belgica 51:80-90.

Brodaty H, Ames D, Boundy KL, Hecker J, Snowdon J, Storey E, Yates MW (2001) Pharmacological treatment of cognitive deficits in Alzheimer's disease. Med J Aust 175:324-327.

Broe M, Shepherd CE, Milward EA, Halliday GM (2001) Relationship between DNA fragmentation, morphological changes and neuronal loss in Alzheimer's disease and dementia with Lewy body. Acta Neuropathol 101:616-624.

Buee L, Bussière T, Buee-Scherrer V, Delacourte A, Hof PR (2000) Tau protein isoforms, phosphorylation and role in degenerative disorders. Brain Res Rev 33:95-130.

Bursch W (2004) Multiple cell death programs: Charon's lifts to Hades. FEMS Yeast Res 5:101-110.

Busciglio J, Gabuzda DH, Matsudaira P, Yankner BA (1993) Generation of beta-amyloid in the secretory pathway in neuronal and nonneuronal cells. Proc Natl Acad Sci U S A 90:2092-2096.

Busciglio J, Lorenzo A, Yeh J, Yanker BA (1995) Beta-amyloid fibrils induce tau phosphorylation and loss of microtubule binding. Neuron 14:879-888.

Bush AI, Pettingell WH, Multhaup G, D Paradis M, Vonsattel JP, Gusella JF, Beyreuther K, Masters CL, Tanzi RE (1994) Rapid induction of Alzheimer Abeta amyloid formation by zinc. Science 265:1464-1467.

Busser J, Geldmacher DS, Herrup K (1998) Ectopic cell cycle proteins predict the sites of neuronal cell death in Alzheimer's disease brain. J Neurosci 18:2801-2807.

Bussière T, Giannakopoulos P, Bouras C, Perl DP, Morrison JH, Hof PR (2003) Progressive degeneration of nonphosphorylated neurofilament protein-enriched pyramidal neurons predicts cognitive impairment in Alzheimer's disease: stereologic analysis of prefrontal cortex area 9. J Comp Neurol 463:281-301.

Bussière T, Bard F, Barbour B, Grajeda H, Guido T, Khan K, Schenk D, Games D, Seubert P, Buttini M (2004) Morphological characterization of thioflavin-S-positive amyloid plaques in transgenic Alzheimer mice and effect of passive Abeta immunotherapy on their clearance. Amer J Pathol 165:987-995.

Buttini M, Orth M, Bellosta S, Akeefe H, Pitas RE, Wyss-Coray T, Mucke L, Mahley RW (1999) Expression of human apolipoprotein E3 or E4 in the brains of Apoe-/- mice: isoform-specific effects on neurodegeneration. J Neurosci 19:4867-4880.

Buttini M, Masliah E, Barbour R, Grajeda H, Motter R, Johnson-Wood K, Khan K, Seubert P, Freedman S, Schenk D, Games D (2005) β-amyloid immunotherapy prevents synaptic degeneration in a mouse model of Alzheimer's disease. J Neurosci 25:9096-9101.

Büki A, Okonkwo DO, Wang KKW, Povlishock JT (2000) Cytochrome c release and caspase activation in traumatic axonal injury. J Neurosci 20:2825-2834.

Caccamo A, Oddo S, Tran LX, LaFerla FM (2007) Lithium reduces tau phosphorylation but not Abeta or working memory deficits in a transgenic model with both plaques and tangles. Amer J Pathol 170:1669-1675.

Calhoun ME, Wiederhold KH, Abramowski D, Phinney AL, Probst A, Sturchler-Pierrat C, Staufenbiel M, Sommer B, Jucker M (1998) Neuron loss in APP transgenic mice. Nature 395:755-756.

Cantarella G, Uberti D, Carsana T, Lombardo G, Bernardini R, Memo M (2003) Neutralization of TRAIL death pathway protects human neuronal cell line from β-amyloid toxicity. Cell Death Differ 10:134-141.

Caraci F, Chisari M, Frasca G, Canonico PL, Battaglia A, Calafiore M, Battaglia G, Bosco P, Nicoletti F, Copani A, Sortino MA (2005) Nicergoline, a drug used for age-dependent cognitive impairment, protects cultured neurons against β-amyloid toxicity. Brain Res 1047:30-37.

Carlson GA, Borchelt DR, Dake A, Turner S, Danielson V, Coffin JD, Eckman C, Meiners J, Nilsen SP, Younkin SG, Hsiao KK (1997) Genetic modification of the phenotypes produced by amyloid precursor proteins overexpression in transgenic mice. Hum Mol Genet 6:1951-1959.

Carrasco J, Adlard P, Cotman C, Quintana A, Penkowa M, Xu F, Van Nostrand WE, Hidalgo J (2006) Metallothionein-I and -III expression in animal models of Alzheimer's disease. Neurosci 143:911-922.

Carroll JC, Rosario ER, Chang L, Stanczyk FZ, Oddo S, LaFerla FM, Pike CJ (2007) Progesterone and estrogen regulate Alzheimer-like neuropathology in female 3xTg-AD mice. J Neurosci 27:13357-13365.

Chaudhuri TK, Paul S (2006) Protein-misfolding diseases and chaperone-based therapeutic approaches. FASEB J 273:1331-1349.

Chauhan NB, Siegel GJ (2003) Intracerebroventricular passive immunization in with anti-Abeta antibody in Tg2576. J Neurosci Res 74:353-357.

Chauhan NB, Siegel GJ, Feinstein DL (2004) Effects of Lovastatin and Pravastatin on amyloid processing and inflammatory response in TgCRND8 brain. Neurochem Res 29:1897-1911.

Chauhan NB, Siegel GJ (2005) Efficacy of anti-Aβ antibody isotypes used for intracerebroventricular immunization in TgCRND8. Neurosci Lett 375:143-147.

Chauhan NB (2007) Intracerebroventricular passive immunization with anti-OligoAβ antibody in TgCRND8. J Neurosci Res 85:451-463.

Check E (2002) Nerve inflammation halts trial for Alzheimer's drug. Nature 415:462.

Cheng C, Zochodne DW (2003) Sensory neurons with activated caspase-3 survive long-term experimental diabetes. Diabetes 52:2362-2371.

Cheng IH, Palop JJ, Esposito LA, Bien-Ly N, Yan F, Mucke L (2004) Aggressive amyloidosis in mice expressing human amyloid peptides with the Arctic mutation. Nature Med 10:1190-1192.

Cheng IH, Scearce-Levie K, Legleiter J, Palop JJ, Gerstein H, Bien-Ly N, Puolivali J, Lesne S, Ashe KH, Muchowski PJ, Mucke L (2007) Accelerating amyloid-β fibrillization reduces oligomer levels and functional deficits in Alzheimer disease mouse models. J Bio Chem 282:23818-23828.

Cherny RA, Legg JT, McLean CA, Fairlie DP, Huang X, Atwood CS, Beyreuther K, Tanzi RE, Masters CL, Bush AI (1999) Aqueous dissolution of Alzheimer's disease Abeta amyloid deposits by biometal depletion. J Bio Chem 274:23223-23228.

Cherny RA, Atwood CS, Xilinas ME, Gray DN, Jones WD, McLean CA, Barnham KJ, Volitakis I, Fraser FW, Kim Y, Huang X, Goldstein LE, Moir RD, Lim JT, Beyreuther K, Zheng H, Tanzi RE, Masters CL, Bush AI (2001) Treatment with a copper-zinc chelator markedly and rapidly inhibits beta-amyloid accumulation in Alzheimer's disease transgenic mice. Neuron 30:665-676.

Cheung ZH, Yip HK, Wu W, So K-F (2003) Axotomy induces cytochrome c release in retinal ganglion cells. NeuroReport 14:279-282.

Chin JY, Knowles RB, Schneider A, Drewes G, Mandelkow E-M, Hyman BT (2000) Microtubule-affinity regulating kinase (MARK) is tightly associated with neurofibrillary tangles in Alzheimer brain: a fluorescence resonance energy transfer study. J Neuropathol Exp Neurol 59:966-971.

Chishti MA, Yang D-S, Janus C, Phinney AL, Horne P, Pearson J, Strome R, Zuker N, Loukides J, French J, Turner S, Lozza G, Grilli M, Kunicki S, Morissette C, Carlson GA, St George-Hyslop P, Westaway D (2001) Early-onset amyloid deposition and

cognitive deficits in transgenic mice expressing a double mutant form of amyloid precursor protein 695. J Bio Chem 276:21562-21570.

Christie RH, Bacskai BJ, Zipfel WR, Williams RM, Kajdasz ST, Webb WW, Hyman BT (2001) Growth arrest of individual senile plaques in a model of Alzheimer's disease observed by in vivo multiphoton microscopy. J Neurosci 21:858-864.

Christie L-A, Su JH, Tu CH, Dick MC, Zhou J, Cotman CW (2007) Differential regulation of inhibitors of apoptosis proteins in Alzheimer's disease. Neurobiol Dis 26:165-173.

Christman CW, Salvant Jr JB, Walker SA, Povlishock JT (1997) Characterization of a prolonged regenerative attempt by diffusely injured axons following traumatic brain injury in adult cat: a light and electron microscopic immunocytochemical study. Acta Neuropathol 94:329-337.

Chuckowree JA, Vickers JC (2003) Cytoskeletal and morphological alterations underlying axonal sprouting after localized transection of cortical neuron axons in vitro. J Neurosci 23:3715-3725.

Chuckowree JA, Dickson TC, Vickers JC (2004) Intrinsic regenerative ability of mature CNS neurons Neuroscientist 10:280-285.

Chung RS, Vickers JC, Chuah MI, West AK (2003) Metallothionein-IIA promotes initial neurite elongation and post injury reactive neurite growth and facilitates healing after focal cortical brain injury. J Neurosci 23:3336-3342.

Chung RS, West AK (2004) A role for extracellular metallothioineins in CNS injury and repair. Neurosci 123:595-599.

Cole GM, Frautschy SA (2006) Docosahexaenoic acid protects from amyloid and dendritic pathology in an Alzheimer's disease mouse model. Nutr Health 18:249-259.

Colucci M, Cammarata S, Assini A, Croce R, Clerici F, Novello C, Mazzella L, Dagnino N, Mariani C, Tanganelli P (2006) The number of pregnancies is a risk factor for Alzheimer's disease. Eur J Neurol 13:1374-1377.

Cotman CW, Su JH (1996) Mechanisms of neuronal death in Alzheimer's disease. Brain Pathol 6:493-506.

Cotman CW (1998) Apoptosis decision cascades and neuronal degeneration in Alzheimer's disease. Neurobiol Aging 19:S29-S32.

Cras P, Kawai M, Lowery D, Gonzalez-DeWhitt P, Greenberg B, Perry G (1991) Senile plaque neurites in Alzheimer disease accumulate amyloid precursor protein. Proc Natl Acad Sci U S A 88:7552-7556.

Cras P, Smith MA, Richey PL, Siedlak SL, Mulvihill P, Perry G (1995) Extracellular neurofibrillary tangles reflect neuronal loss and provide further evidence of extensive protein cross-linking in Alzheimer disease. Acta Neuropathol 89:291-295.

Crouch PJ, White AR, Bush AI (2007) The modulation of metal bio-availability as a therapeutic strategy for the treatment of Alzheimer's disease. FEBS J 274:3775-3783.

Cruz JC, Tsai L-H (2004) Cdk5 deregulation in the pathogenesis of Alzheimer's disease. Trends Mol Med 10:454-458.

Cullen KM, Halliday GM (1998) Neurofibrillary degeneration and cell loss in the nucleus basalis in comparison to cortical Alzheimer pathology. Neurobiol Aging 19:297-306.

Cummings BJ, Cotman CW (1995) Image analysis of β-amyloid load in Alzheimer's disease and relation to dementia severity. Lancet 346:1524-1528.

D'Amore JD, Kajdasz ST, McLellan ME, Bacskai BJ, Stern EA, Hyman BT (2003) In vivo multiphoton imaging of a transgenic mouse model of Alzheimer disease reveals marked thioflavin-S-associated alterations in neurite trajectories. J Neuropathol Exp Neurol 62:137-145.

Das P, Howard V, Loosbrock N, Dickson D, Murphy MP, Golde TE (2003) Amyloid-β immunization effectively reduces amyloid deposition in FcRg-/- knock-out mice. J Neurosci 23:8532-8538.

De Felice FG, Wu D, Lambert MP, Fernandez SJ, Velasco PT, Lacor PN, Bigio EH, Jerecic J, Acton PJ, Shughrue PJ, Chen-Dodson E, Kinney GG, Klein WL (2007) Alzheimer's disease-type neuronal tau hyperphosphorylation induced by Abeta oligomers. Neurobiol Aging Epub ahead of print:doi:10.1016/j.neurobiolaging.2007.02.029.

De la Monte SM, Sohn YK, Wands JR (1997) Correlates of p53- and Fas (CD95)-mediated apoptosis in Alzheimer's disease. J Neurol Sci 152:73-83.

De Leon MJ, Mosconi L, Blennow K, DeSanti S, Zinkowski R, Mehta PD, Pratico D, Tsui W, Saint Louis LA, Sobanska L, Brys M, Li Y, Rich K, Rinne J, Rusinek H (2007) Imaging and CSF studies in the preclinical diagnosis of Alzheimer's disease. Ann N Y Acad Sci 1097:114-145.

DeStrooper B (2003) Aph-1, pen-2 and nicastrin with presenilin generate an active gamma-secretase complex. Neuron 38:9-12.

Del Villar K, Miller CA (2004) Down-regulation of DENN/MADD, a TNF receptor binding protein, correlates with neuronal cell death in Alzheimer's disease brain and hippocampal neurons. Proc Natl Acad Sci U S A 101:4210-4215.

Delatour B, Blanchard V, Pradier L, Duyckaerts C (2004) Alzheimer pathology disorganized cortico-cortical circuitry: direct evidence from a transgenic animal model. Neurobiol Dis 16:41-47.

DeRidder MN, Simon MJ, Siman R, Auberson YP, Raghupathi R, Meany DF (2006) Traumatic mechanical injury to the hippocampus in vitro causes regional caspase-3 and calpain activation that is influenced by NMDA receptor subunit composition. Neurobiol Dis 22:165-176.

Descarries L, Aznavour N, Hamel E (2005) The acetylcholine innervation of cerebral cortex: new data on its normal development and its fate in the hAPPSW,IND mouse model of Alzheimer's disease. J Neural Transm 112:149-162.

Desjardins P, Ledoux S (1998) Expression of ced-3 and ced-9 homologs in Alzheimer's disease cerebral cortex. Neurosci Lett 244:69-72.

DeWitt DA, Silver J (1996) Regenerative failure: a potential mechanism for neuritic dystrophy in Alzheimer's disease. Exp Neurol 142:103-110.

Dickenstein DL, Biron KE, Ujie M, Pfeifer CG, Jeffries AR, Jefferies WA (2006) Aβ peptide immunization restores blood-brain barrier integrity in Alzheimer disease. FASEB J 20:426-433.

Dickson DW, Farlo J, Davies P, Crystal H, Fuld PY, en S-HC (1988) Alzheimer's disease a double labelling immunohistochemical study of senile plaques. Amer J Pathol 132:86-101.

Dickson TC, Saunders HL, Vickers JC (1997) Relationship between apoplipoprotein E and the amyloid deposits and dystrophic neurites of Alzheimer's disease. Neuropath App Neurobiol 23:483-491.

Dickson TC, King CE, McCormack GH, Vickers JC (1999) Neurochemical diversity of dystrophic neurites in the early and late stages of Alzheimer's disease. Exp Neurol 156:100-110.

Dickson TC, Adlard PA, Vickers JC (2000) Sequence of cellular changes following localized axotomy to cortical neurons in glia-free cultures. J Neurotrauma 17:1095-1103.

Dickson TC, Vickers JC (2001) The morphological phenotype of beta-amyloid plaques and associated neuritic changes in Alzheimer's disease. Neurosci 105:99-107.

Dickson TC, Chuckowree JA, Chuah MI, West AK, Vickers JC (2005) Novel Alzheimer's disease pathology reflects variable neuronal vulnerability and demonstrates the role of β-amyloid plaques in neurodegeneration. Neurobiol Dis 18:286-295.

Diez M, Danner S, Frey P, Sommer B, Staufenbiel M, Wiederhold KH, Hokfelt T (2003) Neuropeptide alterations in the hippocampal formation and cortex of transgenic mice overexpressing beta-amyloid precursor protein (APP) with the Swedish double mutation (APP23). Neurobiol Dis 14:579-594.

Dodart J-C, Bales KR, Paul SM, Ungerer A (1999) Behavioural disturbances in transgenic mice overexpressing the V717F β-amyloid precursor protein. Behav Neurosci 113:982-990.

Dodart JC, Bales KR, Gannon KS, Greene SJ, DeMattos RB, Mathis C, DeLong CA, Wu S, Wu X, Holtzman DM, Paul SM (2002) Immunization reverses memory deficits without reducing brain Abeta burden in Alzheimer's disease model. Nat Neurosci 5:452-457.

Dodart J-C, Marr RA, Koistinaho M, Gregersen BM, Malkani S, Verma IM, Paul SM (2005) Gene delivery of human apolipoprotein E alters brain Aβ burden in a mouse model of Alzheimer's disease. Proc Natl Acad Sci U S A 102:1211-1216.

Dodel RC, Yu D, Depboylu C, Hampel H, Frolich L, Haag A, Hemmeter U, Aulsen S, Teipel SJ, Breetschneider S, Spottke A, Nolker C, Moller HJ, Wei X, Farlow M,

Sommer N, Oertel WH (2004) Intravenous immunoglobulins containing antibodies against β-amyloid for the treatment of Alzheimer's disease. J Neurol Neurosurg Psych 75:1472-1474.

Domañiksa-Janik K, Buzañska L, Duzniewsdk J, Kolowska H, Sarnowska A, Zablocka B (2004) Neuroprotection by cyclosporin A following transient brain ischemia correlates with the inhibition of the early efflux of cytochrome c to cytoplasm. Mol Brain Res 121:50-59.

Dong H, Martin MV, Chambers S, Csernansky CA (2007) Spatial relationship between synapse loss and β-amyloid deposition in Tg2576 mice. J Comp Neurol 500:311-321.

Dragunow M, Faull RLM, Lawlor P, E.J. B, Singleton K, Walker EB, Mee E (1995) In situ evidence for DNA fragmentation in Huntington's disease striatum and Alzheimer's disease temporal lobes. NeuroReport 6:1053-1057.

Duan H, Dixit VM (1997) RAIDD is a new 'death' adaptor molecule. Nature 385:86-89.

Dudal S, Krzywkowski P, Paquette J, Morissette C, Lacombe D, Tremblay P, Gervais F (2004) Inflammation occurs early during the Aβ deposition process in TgCRND8 mice. Neurobiol Aging 25:861-871.

Duff K, Eckman C, Zehr C, Yu X, Prada C-M, Perez-Tur J, Hutton M, Buee L, Harigaya Y, Yager D, Morgan D, Gordon MN, Holcomb L, Refolo L, Zenk B, Hardy J, Younkin S (1996) Increased amyloid-β42(43) in brains of mice expressing mutant presenilin 1. Nature 383:710-713.

Duff K, Knight H, Refolo LM, Sanders S, Yu X, Picciano M, Malester B, Hutton M, Adamson J, Goedert M, Burki K, Davies P (2000) Characterization of pathology in transgenic mice over-expressing human genomic and cDNA tau transgenes. Neurobiol Dis 7:87-98.

Duguid JR, Dohmont CW, Liu NG, Tourtellotte WW (1989) Changes in brain gene expression shared by scrapie and Alzheimer's disease. Proc Natl Acad Sci U S A 86:7260-7264.

Engel T, Goni-Oliver P, Lucas JJ, Avila J, Hernandez F (2006) Chronic lithium administration to FTDP-17 tau and GSK-3beta overexpressing mice prevents tau

hyperphosphorylation and neurofibrillary tangle formation, but pre-formed neurofibrillary tangles do not revert. J Neurochem 99:1445-1455.

Engelhart MJ, Geerlings MI, Ruitenberg A, Van Swieten JC, Hofman A, Witteman JC, Breteler MM (2002) Dietary intake of antioxidant and risk of Alzheimer's disease. JAMA 287:3223-3229.

Engidawork E, Gulesserian T, Seidl R, Cairns N, Lubec G (2001) Expression of apoptosis related proteins: RAIDD, ZIP kinase, Bim/BOD, p21, Bax, Bcl-2 and NF-kB in brains of patients with Down syndrome. In: Protein expression in Down Syndrome Brain (Lubec G, ed), pp 181-192. New York: Springer-Verlag/Wien.

Eriksen JL, Sagi SA, Smith TE, Weggen S, Das P, McLendon DC, Ozols VV, Jessing KW, Zavitz KH, Koo EH, Golde TE (2003) NSAIDs and enantiomers of flurobiprofen target γ-secretase and lower Aβ42 in vivo. J Clin Invest 112:440-449.

Estus S, Tucker HM, Van Rooyen C, Wright S, Brigham EF, Wogulis M, Rydel RE (1997) Aggregated amyloid-β protein induces cortical neuronal apoptosis and concomitant "apoptotic" pattern of gene induction. J Neurosci 17:7736-7745.

Ferreira A, Lu Q, Orecchio L, Kosik KS (1997) Selective phosphorylation of adult tau isoforms in mature hippocampal neurons exposed to fibrillar Abeta. Mol Cell Neurosci 9:220-234.

Ferrer I, Soriano E, Tuñón T, Fonseca M, Guionnet N (1991) Parvalbumin immunoreactive neurons in normal human temporal neocortex and in patients with Alzheimer's disease. J Neurol Sci 106:135-141.

Ferrer I, Puig B, Krupinski J, Carmona M, Blanco R (2001) Fas and Fas ligand expression in Alzheimer's disease. Acta Neuropathol 102:121-131.

Ferrer I, Rovira MB, Sanchez Guerra ML, Rey MJ, Costa-Jussa F (2004) Neuropathology and pathogenesis of encephalitis following amyloid-β immunization in Alzheimer's disease. Brain Pathol 14:11-20.

Finkelstein DI, Wilkins S, Robb EL, Bush AI, Cherny RA (2007) Soluble Aβ in the brains of conscious Tg2576 mice is rapidly reduced following treatment with 8-Hydroxy quinoline drugs: in vivo brain microdialysis as a tool for drug development. International Brain Research Organisation World Congress of Neuroscience: Melbourne, Australia.

Forloni G, Chiesa R, Smiroldo S, Verga L, Salmona M, Tagliavini F, Angeretti N (1993) Apoptosis mediated neurotoxicity induced by chronic application of β-amyloid fragment 25-35. NeuroReport 4:523-526.

Fraser PE, Levesque L, McLachlan DR (1993) Biochemistry of Alzheimer's disease amyloid plaques. Clin Biochem 26:339-349.

Frautschy SA, Yang F, Irizarry M, Hyman B, Saido TC, Hsiao K, Cole G (1998) Microglial response to amyloid plaques in APPsw transgenic mice. Amer J Pathol 152:307-317.

Frenkel D, Maron R, Burt DS, Weiner HL (2005) Nasal vaccination with a proteosome-based adjuvant and glatiramer acetate clears β-amyloid in a mouse model of Alzheimer disease. J Clin Invest 115:2423-2433.

Freund-Levi Y, Eriksdotter-Jonhagen M, Cederholm T, Basun H, Faxen-Irving G, Garlind A, Vedin I, Vessby B, Wahlund L-O, Palmblad J (2006) ω-3 fatty acid treatment in 174 patients with mild to moderate Alzheimer's disease: OmegAD study. Arch Neurol 63:1402-1408.

Fukumoto H, Asami-Odaka AS, Uzuki N, Iwatsubo T (1996) Association of Aβ40-positive senile plaques with microglial cells in the brains of patients with Alzheimer's disease and in non-demented aged individuals. Neurodegeneration 5:13-17.

Fukumoto H, Rosene DL, Moss MB, Raju S, Hyman BT, Irizarry MC (2004) β-secretase activity increases with aging in human, monkey and mouse brain. Amer J Pathol 164:719-725.

Fukutani Y, Kobayashi K, Nakamura I, Watanabe K, Isaki K, Cairns NJ (1995) Neurons, intracellular and extracellular neurofibrillary tangles in subdivisions of the hippocampal cortex in normal ageing and Alzheimer's disease. Neurosci Lett 200:57-60.

Fumagalli F, Racagni G, Riva MA (2006) The expanding role of BDNF: a therapeutic target for Alzheimer's disease. Pharmacogenomics J 6:8-15.

Furukawa K, Sopher BL, Rydel RE, Begley JG, Pham DG, Martin GM, Fox M, Mattson MP (1996) Increased activity-regulating and neuroprotective efficacy of α-secretase-

derived secreted amyloid precursor protein conferred by a C-terminal heparin-binding domain. J Neurochem 67:1882-1896.

Gamblin TC, Chen F, Zambrano A, Abraha A, Lagalwar S, Guillozet AL, Lu M, Fu Y, Garcia-Sierra F, LaPointe N, Miller R, Berry RW, Binder LI, Cryns VL (2003) Caspase cleavage of tau: linking amyloid and neurofibrillary tangles in Alzheimer's disease. Proc Natl Acad Sci U S A 100:10032-10037.

Games D, Adams D, Alessandrini R, Barbour B, Bethelette P, Blackwell C, Carr T, Gillespie F, Guido T, Hagopian S, Johnson-Wood K, Khan K, Lee M, Lelbowitz P, Leiberburg I, Little S, Masliah E, McConiogue L, Montoya-Zavaia M, Mucke L, Paganini L, Penniman E, Power M, Schenk D, Seubert P, Snyder B, Soriano F, Tan H, Vitale J, Wadesworth S, Wolozin B, Zhao J (1995) Alzheimer-type neuropathology in transgenic mice overexpressing V717F β-amyloid precursor protein. Nature 373:523-527.

Garcia-Alloza M, Dodwell SA, Meyer-Luehmann M, Hyman BT, Bacskai BJ (2006) Plaque-derived oxidative stress mediates distorted neurite trajectories in the Alzheimer's disease mouse model. J Neuropathol Exp Neurol 65:1082-1089.

Gastard MC, Troncoso JC, Koliatsos VE (2003) Caspase activation in the limbic cortex of subjects with early Alzheimer's disease. Ann Neurol 54:393-398.

Gestwicki JE, Crabtree GR, Graef IA (2004) Harnessing chaperones to generate small-molecule inhibitors of amyloid-β aggregation. Science 306:865-868.

Giannakopoulos P, Kovari E, Savioz A, De Bilbao F, Dubois-Dauphin M, Hof PR, Bouras C (1999) Differential distribution of presenilin-1, Bax, Bcl-XL in Alzheimer's disease and frontotemporal dementia. Acta Neuropathol 98:141-149.

Giannakopoulos P, Herrmann FR, Bussière T, Bouras C, Kovari E, Perl DP, Morrison JH, Gold G, Hof PR (2003) Tangle and neuron numbers, but not amyloid load, predict cognitive status in Alzheimer's disease. Neurology 60:1495-1500.

Gibson GE, Haroutunian V, Zhang BM, Park LCH, Shi Q, Lesser M, Mohs RC, Sheu RK-F, Blass JP (2000) Mitochondrial damage in Alzheimer's disease varies with apolipoprotein E genotype. Ann Neurol 48:297-303.

Gilman S, Koller M, Black RS, Jenkins L, Griffith SG, Fox NC, Eisner L, Kirby L, Rovira B, Forette F, Orgogozo J-M (2005) Clinical effects of Aβ immunization (AN1792) in patients with AD in an interrupted trial. Neurology 64:1553-1562.

Giralt M, Penkowa M, Lago N, Molinero A, Hidalgo J (2002) Metallothionein-1+2 protect the CNS after a focal brain injury. Exp Neurol 173:114-128.

Gold G, Kovari E, Corte G, Hermann FR, Canuto A, Bussière T, Hof PR, Bouras C, Giannakopoulos P (2001) Clinical validity of Aβ-protein deposition staging in brain aging and Alzheimer disease. J Neuropathol Exp Neurol 60:946-952.

Gomez-Isla T, Price JL, McKeel Jr DW, Moris JC, Growdon JH, Hyman BT (1996) Profound loss of layer III entorhinal cortex neurons occurs in very mild Alzheimer's disease. J Neurosci 16:4491-4500.

Gomez-Isla T, Hollister R, West H, Mui S, Growdon JH, Petersen RC, Parisi JE, Hyman BT (1997) Neuronal loss correlates with but exceeds neurofibrillary tangles in Alzheimer's disease. Ann Neurol 41:17-24.

Gomez-Ramos P, Mufson EJ, Moran MA (2001) Apolipoprotein E immunoreactivity in neurons and neurofibrillary degeneration of aged non-demented and Alzheimer's disease patients. Microsc Res Tech 55:48-58.

Goodman Y, Mattson MP (1994) Secreted forms of β-amyloid precursor protein protect hippocampal neurons against amyloid β-peptide-induced oxidative injury. Exp Neurol 128:1-12.

Gordon RK, Nigam SV, Weitz JA, Dave JR, Doctor BP, Ved HS (2001) The NMDA receptor ion channel: a site for binding of huperine A. J App Toxicol 21:S47-S51.

Gotz J, Chen F, Barmettler R, Nitsch RM (2001) Tau filament formation in transgenic mice expressing P301L tau. J Biol Chem 276:529-534.

Gotz J, Streffer JR, David D, Schild A, Hoerndli F, Pennanen L, Kurosinski P, Chen F (2004) Transgenic animal models of Alzheimer's disease and related disorders: histopathology, behaviour and therapy. Mol Phsych 9:664-683.

Green KN, Martinez-Coria H, Khashwji H, Hall EB, Yurko-Mauro KA, Ellis L, LaFerla FM (2007) Dietary docosahexaenoic acid and docosapentaenoic acid ameliorate amyloid-β and tau pathology via a mechanism involving presenilin 1 levels. J Neurosci 27:4385-4395.

Grundman M, Capparelli E, Kim HT, Morris JC, Farlow M, Rubin EH, Heidebrink J, Hake A, Ho G, Schultz AN, Schafer K, Houston W, Thomas R, Thal LJ (2003) A multicenter, randomized, placebo controlled, multiple-dose, safety and pharmacokinetic study of AIT-082 (Neotrofin) in mild Alzheimer's disease patients. Life Sci 73:539-553.

Grutzendler J, Helmin K, Tsai J, Wen-Biao G (2007) Various dendritic abnormalities are associated with fibrillar amyloid deposits in Alzheimer's disease. Ann N Y Acad Sci 1097:30-39.

Gschwind M, Huber G (1995) Apoptotic cell death induced by β-amyloid1-42 peptides is cell type dependent. J Neurochem 65:292-300.

Guo Q, Fu W, Sopher BL, Miller MW, Ware CB, Martin GM, Mattson MP (1999) Increased vulnerability of hippocampal neurons to excitotoxic necrosis in presenilin-1 mutant knock-in mice. Nature 5:101-106.

Guo H, Albrecht S, Bourdeau M, T. P, Bergeron C, LeBlanc AC (2004) Active caspase-6 and caspase-6-cleaved tau in neuropil threads, neuritic plaques, and neurofibrillary tangles of Alzheimer's disease. Amer J Pathol 165:523-531.

Gutala RV, Reddy PH (2004) The use of real time RT-PCR in a gene expression study of Alzheimer's disease post-mortem brains. J Neurosci Methods 132:101-107.

Haass C, Selkoe DJ (1993) Cellular processing of beta-amyloid precursor protein and the genesis of amyloid beta-peptide. Cell 75:1039-1042.

Hara H, Monsonego A, Yuasa K, Adachi K, Xiao X, Takeda S, Takahashi K, Weiner HL, Tabira T (2004) Development of a safe oral Aβ vaccine using recombinant adeno-associated virus vector for Alzheimer's disease. J Alzheimer's Dis 6:483-488.

Hardy J, Duff K, Hardy KG, Perez-Tur J, Hutton M (1998) Genetic dissection of Alzheimer's disease and related dementias: amyloid and its relationship to tau. Nat Neurosci 1:355-358.

Harel NY, Strittmatter SM (2006) Can regenerating axons recapitulate developmental guidance during recovery from spinal cord injury? Nat Rev Neurosci 7:603-616.

Hartley DM, Walsh DM, Ye CP, Diehl T, Vazquez S, Vassilev PM, Teplow DB, Selkoe DJ (1999) Protofibrillar intermediates of amyloid β-protein induce acute

electrophysiological changes and progressive neurotoxicity in cortical neurons. J Neurosci 19:8876-8884.

Hartman RE, Izumi Y, Bales KR, Paul SM, Wozniak DF, Holtzman DM (2005) Treatment with an amyloid-β antibody ameliorates plaque load, learning deficits, and hippocampal long-term potentiation in a mouse model of Alzheimer's disease. J Neurosci 25:6213-6220.

Hartman RE, Shah A, Fagan AM, Schwetye KE, Parsadanian M, Schulman RN, Finn MB, Holtzman DM (2006) Pomegranate juice decreases amyloid load and improves behaviour in a mouse model of Alzheimer's disease. Neurobiol Dis 24:506-515.

Hartmann T, Bieger SC, Brühl B, Tienari PJ, Ida N, Allsop D, Roberts GW, Masters CL, Dotti CG, Unsicker K, Beyreuther K (1997) Distinct sites of intracellular production of Alzheimer's disease Aβ40/42 amyloid peptides. Nat Med 3:1016-1020.

He Y, Sun S-H, Chen R-W, Guo Y-J, He X-W, Huang L, Chen Z-H, Shi K, Zhu W-J (2005) Effects of epitopes combination and adjuvants on immune responses to anti-Alzheimer disease DNA vaccines in mice. Alzheimer Dis Assoc Disord 19:171-177.

Hidalgo J, Aschner M, Zatta P, Vasak M (2001) Roles of the metallothionein family of protein in the central nervous system. Brain Res Bull 55:133-145.

Higgins GA, Jacobsen H (2003) Transgenic mouse models of Alzheimer's disease: phenotype and application. Behav Pharm 14:419-428.

Hirai K, Aliev G, Nunomura A, Fujioka H, Russell RL, Atwood CS, Johnson AB, Kress Y, Vinters HV, Tabaton M, Shimohama S, Cash AD, Siedlak SL, Harris PLR, Jones PK, Peterson RB, Perry G, Smith MA (2001) Mitochondrial abnormalities in Alzheimer's disease. J Neurosci 21:3017-3023.

Ho L, Qin W, Pompl PN, Xiang Z, Wang J, Zhao Z, Peng Y, Cambareri G, Rocher A, Mobbs CV, Hof PR, Pasinetti GM (2004) Diet-induced insulin resistance promotes amyloidosis in a transgenic mouse model of Alzheimer's disease. FASEB J 18:902-904.

Hock C, Konietzko U, Streffer JR, Tracy J, Signorell A, Muller-Tillmanns B, Lemke Y, Henke K, Moritz E, Garcia E, Wollmer MA, Umbricht D, De Quervain DJF, Hofmann M, Madalena A, Papassotiropoulos A, Nitsch RM (2003) Antibodies

against β-amyloid slow cognitive decline in Alzheimer's disease. Neuron 38:547-554.

Hof PR, Cox K, Morrison JH (1990) Quantitative analysis of a vulnerable subset of pyramidal neurons in Alzheimer's disease: I. Superior and inferior temporal cortex. J Comp Neurol 301:44-54.

Hof PR, Cox K, Young WG, Celio MR, Rogers J, Morrison JH (1991a) Parvalbumin-immunoreactive neurons in the neocortex are resistant to degeneration in Alzheimer's disease. J Neuropathol Exp Neurol 50:451-462.

Hof PR, Morrison JH (1991b) Neocortical neuronal subpopulations labeled by a monoclonal antibody to calbindin exhibit differential vulnerability in Alzheimer's disease. Exp Neurol 111:293-301.

Hof PR, Bouras C, Buee L, Delacourte A, Perl DP, Morrison JH (1992) Differential distribution of neurofibrillary tangles in the cerebral cortex of dementia publistica and Alzheimer's disease. Acta Neuropathol 85:23-30.

Hof PR, Nimchinsky EA, Celio MR, Bouras C, Morrison JH (1993) Calretinin-immunoreactive neocortical interneurons are unaffected in Alzheimer's disease. Neurosci Lett 152:145-148.

Hof PR (1997) Morphological and neurochemical characteristics of the vulnerable neurons in brain aging and Alzheimer's disease. Eur Neurol 37:71-81.

Hogervorst E, Bandelow S, Combrinck M, Smith AD (2004) Low free testosterone is an independent risk factor for Alzheimer's disease. Exp Gerontol 39:1633-1639.

Hoglund K, Syversen S, Lewczuk P, Wallin A, Wiltfang J, Blennow K (2005) Statin treatment and a disease-specific pattern of β-amyloid peptides in Alzheimer's disease. Exp Brain Res 164:205-214.

Holcomb L, Gordon MN, McGowan E, Yu X, Benkovic S, Jantzen P, Wright K, Saad I, Mueller R, Morgan D, Sanders S, Zehr C, O'Campo K, Hardy J, Prada CM, Eckman C, Younkin S, Hsiao K, Duff K (1998) Accelerated Alzheimer-type phenotype in transgenic mice carrying both mutant amyloid precursor protein and presenilin 1 transgenes. Nat Med 4:97-100.

Holcomb LA, Gordon MN, Jantzen P, Hsiao K, Duff K, Morgan D (1999) Behavioral changes in transgenic mice expressing both amyloid precursor protein and

presenilin-1 mutations: lack of association with amyloid deposits. Behavior Genetics 29:177-185.

Holtzman DM, Pitas RE, Kilbridge H, Nathan B, Mahley RW, Bu G, Schwartz AL (1995) Low density lipoprotein receptor-related protein mediates apoplipoprotein E-dependent neurite outgrowth in a central nervous system-derived neuronal cell line. Proc Natl Acad Sci U S A 92:9480-9484.

Holtzman DM, Fagan AM, Mackey B, Tenkova T, Sartorius L, Paul SM, Bales K, Ashe KH, Irizarry MC, Hyman BT (2000) Apolipoprotein E facilitates neuritic and cerebrovascular plaque formation in an Alzheimer's disease model. Ann Neurol 47:739-747.

Horikoshi Y, Mori T, Maeda M, Kinoshita N, Sato K, Yamaguchi H (2004) Aβ N-terminal-end specific antibody reduced β-amyloid in Alzheimer-model mice. Biochem Biophys Res Comm 325:384-387.

Horrocks LA, Farooqui AA (2004) Docosahexaenoic acid in the diet: its importance in maintenance and restoration of neural membrane function. Prostaglandins Leukot Essent Fatty Acids 70:361-372.

Hoshi M, Sato M, Matsumoto S, Noguchi A, Yasutake K, Yoshida N, Sato K (2003) Spherical aggregates of β-amyloid (amylospheroid) show high neurotoxicity and activate tau protein kinase I/glycogen synthase kinase-3β. Proc Natl Acad Sci U S A 100:6370-6375.

Hou XE, Lundmark K, Dahlstrom AB (1998) Cellular reactions to axotomy in rat superior cervical ganglia includes apoptotic cell death. J Neurocytol 27:441-451.

Houlden H, Baker M, McGowan E, Lewis P, Hutton M, Crook R, Wood NW, Kumar-Singh S, Geddes J, Swash M, Scaravilli F, Holton JL, Lashley T, Tomita T, Hashimoto T, Verkkoniemi A, Kalimo H, Somer M, Paetau A, Martin JJ, Van Broeckhoven C, Golde T, Hardy J, Halita M, Revesz T (2000) Variant Alzheimer's disease with spastic paraparesis and cotton wool plaques is caused by PS-1 mutations that lead to exceptionally high amyloid-beta concentrations. Ann Neurol 48:806-808.

Howlett DR, Jennings KH, Lee DC, Clark MSG, Brown F, Wetzel R, Wood SJ, Camilleri P, Roberts GW (1995) Aggregation state and neurotoxic properties of Alzheimer beta-amyloid peptide. Neurodegeneration 4:23-32.

Howlett DR, Richardson JC, Austin A, Parsons AA, Bate ST, Davies DC, Gonzalez MI (2004) Cognitive correlates of Aβ deposition in male and female mice bearing amyloid precursor protein and presenilin-1 mutant transgenes. Brain Res 1017:130-136.

Hsiao K, Chapman P, Nilsen S, Eckman C, Harigaya Y, Younkin S, Yang F, Cole G (1996) Correlative memory deficits, Aβ elevation, and amyloid plaques in transgenic mice. Science 274:99-102.

Huang X, Atwood CS, Moir RD, Hartshorn MA, Vonsattel JP, Tanzi RE, Bush AI (1997) Zinc-induced Alzheimer's Abeta1-40 aggregation is mediated by conformational factors. J Biol Chem 272:26464-26470.

Huang X, Cuajungco MP, Atwood CS, Hartshorn MA, Tyndall JD, Hanson GR, Stokes KC, Leopold M, Multhaup G, Goldstein LE, Scarpa RC, Saunders AJ, Lim J, Moir RD, Glabe C, Bowden EF, Masters CL, Fairlie DP, Tanzi RE, Bush AI (1999) Cu(II) potentiation of Alzheimer Abeta neurotoxicity. Correlation with cell-free hydrogen peroxide production and metal reduction. J Biol Chem 274:37111-37116.

Huang S-M, Mouri A, Kokubo H, Nakajima R, Suemoto T, Higuchi M, Staufenbiel M, Noda Y, Yamaguchi H, Nabeshima T, Saido TC, Iwata N (2006) Neprilysin-sensitive synapse-associated amyloid-β peptide oligomers impair neuronal plasticity and cognitive function. J Biol Chem 281:17941-17951.

Hussain I, Hawkins J, Harrison D, Hille C, Wayne G, Cutler L, Buck T, Walter D, Demont E, Howes C, Naylor A, Jeffrey P, Gonzalez MI, Dingwall C, Michel A, Redshaw S, Dacis JB (2007) Oral administration of a potent and selective non-peptidic BACE-1 inhibitor decreases β-cleavage of amyloid precursor protein and amyloid-β production in vivo. J Neurochem 100:802-809.

Hwang DY, Chae KR, Kang TS, Hwang JH, Lim CH, Kang HK, Goo JS, Lee MI, Lim HJ, Min SH, Cho JY, Hong JT, Song CW, Paik SG, Cho JS, Kim YK (2002) Alterations in behavior, amyloid-β-42, caspase-3, and Cox-2 in mutant PS2 transgenic mouse model of Alzheimer's disease. FASEB J 16:805-813.

Hyde LA, Kazdoba TM, Grilli M, Lozza G, Brusa R, Zhang Q, Wong GT, McCool ME, Zhang L, Parker EM, Higgins GA (2005) Age-progressing cognitive impairments and neuropathology in transgenic CRND8 mice. Behav Brain Res 160:344-355.

Iqbal K, Alonso ADC, Chen S, Chohan MO, El-Akkad E, Gong C-X, Khatoon S, Li B, Liu F, Rahman A, Tanimukai H, Grundke-Iqbal I (2005) Tau pathology in Alzheimer disease and other tauopathies. Biochimica et Biophysica Acta 1793:198-210.

Irizarry MC, McNamara M, Fredorchak K, Hsaio K, Hyman BT (1997) APPSW transgenic mice develop age-related Aβ deposits and neuropil abnormalities, but no neuronal loss in CA1. J Neuropathol Exp Neurol 56:965-973.

Ivins KJ, Bui ETN, Cotman CW (1998) β-amyloid induces local neurite degeneration in cultured hippocampal neurons: evidence for neuritic apoptosis. Neurobiol Dis 5:365-378.

Ivins KJ, Thornton PL, Rohn TT, Cotman CW (1999) Neuronal apoptosis induced by β-amyloid is mediated by caspase-8. Neurobiol Dis 6:440-449.

Iwatsubo T, Odaka A, Suzuki N, Mizusawa H, Nukina N, Ihara Y (1994) Visualization of Aβ42(43) and Aβ40 in senile plaques with end-specific Aβ monoclonals: evidence that an initially deposited species is Aβ42(43). Neuron 13:45-53.

Jacobsen JS, Wu C-C, Redwine JM, Comery TA, Arias R, Bowlby M, Martone R, Morrison JH, Pangalos MN, Reinhart PH, Bloom FE (2006) Early-onset behavioral and synaptic deficits in a mouse model of Alzheimer's disease. Proc Natl Acad Sci U S A 103:5161-5166.

Jang J-H, Surh Y-J (2002) β-amyloid induces oxidative DNA damage and cell death through activation of c-jun terminal kinase. Ann N Y Acad Sci 973:228-236.

Jang J-H, Surh Y-J (2005) β-amyloid-induced apoptosis is associated with cyclooxygenase-2 up-regulation via the mitogen-activated protein kinase-NF-kB signalling pathway. Free Rad Biol Med 38:1604-1613.

Janssen JC, Lantos PL, Fox NC, Harvey RJ, Beck J, Dickinson A, Campbell TA, Collinge J, Hanger DP, Cipolotti L, Stevens JM, Rosser MN (2001) Autopsy-confirmed familial early-onset Alzheimer disease caused by the L153V presenilin 1 mutation. Arch Neurol 58:953-958.

Janus C, Pearson J, McLaurin J, Mathews PM, Jiang Y, Schmidt S, Chishti MA, Home P, Heslin D, French J, Mount HTJ, Nixon RA, Mercken M, Bergeron C, Fraser PE, St George-Hyslop P, Westaway D (2000) Aβ peptide immunization reduces behavioral impairment and plaques in a model of Alzheimer's disease. Nature 408:979-982.

Jellinger KA, Stadelmann C (2000) Mechanisms of cell death in neurodegenerative disorders. J Neural Transm Suppl 59:95-114.

Jellinger KA, Stadelmann C (2001) Problems of cell death in neurodegeneration and Alzheimer's disease. J Alzheimer's Dis 3:31-40.

Jellinger KA (2006) Challenges in neuronal apoptosis. Curr Alzheimer Res 3:377-391.

Jensen MT, Mottin MD, Cracchiolo JR, Leighty RE, Arendash GW (2005) Lifelong immunization with human β-amyloid (1-42) protects Alzheimer's transgenic mice against cognitive impairment throughout aging. Neurosci 130:667-684.

Ji Y, Gong Y, Gan W, Beach T, Holtzman DM, Wisniewski T (2003) Apolipoprotein E isoform-specific regulation of dendritic spine morphology in apolipoprotein E transgenic mice and Alzheimer's disease patients. Neurosci 122:305-315.

Jiang H, Luo X, Bai D (2003) Progress in clinical, pharmacological, chemical and structural biological studies of huperzine A: a drug of traditional Chinese medicine origin for the treatment of Alzheimer's disease. Curr Med Chem 10:2231-2252.

Jick H, Zornberg GL, Jick SS, Seshadri S, Drachman DA (2000) Statins and the risk of dementia. Lancet 356:1627-1631.

Jimenez-Huete A, Alfonso P, Soto C, Albar JP, Rabano A, Ghiso J, Frangione B, Mendez E (1998) Antibodies directed to the carboxyl terminus of amyloid β-peptide recognize sequence epitopes and distinct immunoreactive deposits in Alzheimer's disease brain. Alzheimer's Reports 1:41-48.

Johnson GV, Jenkins SM (1999) Tau protein in normal and Alzheimer's disease brain. J Alzheimer's Dis 1:307-328.

Jordan J, Cena V, Prehn JH (2003) Mitochondrial control of neuron death and its role in neurodegenerative disorders. J Physiol Biochem 59:129-141.

Joseph J, Shukitt-Hale B, Denisova NA, Martin A, Perry G, Smith MA (2001) Copernicus revisited: amyloid beta in Alzheimer's disease. Neurobiol Aging 22:131-146.

Kaasik A, Vassiljev V, Poldoja E, Kalda A, Zharkovsky A (1999) Do nuclear condensation or fragmentation and DNA fragmentation reflect the mode of neuronal death? NeuroReport 10:1937-1942.

Kampers T, Pangalos M, Geerts H, Wiech H, Mandelkow E (1999) Assembly of paired helical filaments from mouse tau: implications for the neurofibrillary pathology in transgenic mouse models for Alzheimer's disease. FEBS Letters 451:39-44.

Kang HJ, Yoon WJ, Moon GJ, Kin DY, Sohn S, Kwon HJ, Gwag BJ (2005) Caspase-3-mediated cleavage of PHF-1 tau during apoptosis irrespective of excitotoxicity and oxidative stress: an implication to Alzheimer's disease. Neurobiol Dis 18:450-458.

Kawarabayashi T, Younkin LH, Saido TC, Shoji M, Ashe KH, Younkin SG (2001) Age-dependent changes in brain, CSF, and plasma amyloid-β protein in the Tg2576 transgenic mouse model of Alzheimer's disease. J Neurosci 21:372-381.

Kawasumi M, Chiba T, Yamada M, Miyamae-Kaneko M, Matsuoka M, Nakahara J, Tomita T, Iwatsubo T, Kato S, Aiso S, Nishimoto I, Kouyama K (2004) Targeted introduction of V642I mutation in amyloid precursor protein gene causes functional abnormality resembling early stage of Alzheimer's disease in aged mice. Eur J Neurosci 19:2826-2838.

Keil U, Bonert A, Marques CA, Scherping I, Weyermann J, Strosznajder JB, Muller-Spahn F, Haass C, Czech C, Pradier L, Muller WE, Eckert A (2004) Amyloid β-induced changes in nitric oxide production and mitochondrial activity lead to apoptosis. J Biol Chem 279:50310-50320.

Kelly PH, Bondolfi L, Hunziker D, Schlecht H-P, Carver K, Maguire E, Abramowski D, Weiederhold K-H, Sturchler-Pierrat C, Jucker M, Bergmann R, Staufenbiel M, Sommer B (2003) Progressive age-related impairment of cognitive behavior in APP23 transgenic mice. Neurobiol Aging 24:365-378.

Kim H-D, Kong F-K, Cao Y, Lewis TL, Kim H, Tang DC, Fukuchi K-I (2004) Immunization of Alzheimer model mice with adenovirus vectors encoding amyloid

β-protein and GM-CSF reduces amyloid load in the brain. Neurosci Lett 370:218-223.

Kim J, Onstead L, Randle S, Price R, Smithson L, Zwizinski C, Dickson DW, Golde T, McGowan E (2007) Aβ40 inhibits amyloid deposition in vivo. J Neurosci 27:627-633.

King CE, Jacobs I, Dickson TC, Vickers JC (1997) Physical damage to rat cortical axons mimics early Alzheimer's neuronal pathology. NeuroReport 8:1663-1665.

King CE, Adlard PA, Dickson TC, Vickers JC (2000) Neuronal response to physical injury and its relationship to the pathology of Alzheimer's disease. Clin Exp Pharm Phys 27:548-552.

King CE, Canty AJ, Vickers JC (2001) Alterations in neurofilaments associated with reactive brain changes and axonal sprouting following acute physical injury to the rat neocortex. Neuropathol Appl Neurobiol 27:115-126.

King AE, Chung RS, Vickers JC, Dickson TC (2006) Localization of glutamate receptors in developing cortical neurons in culture and relationship to susceptibility to excitotoxicity. J Comp Neurol 498:277-294.

Kirkcaldie MT, Dickson TC, King CE, Grasby D, Riederer BM, Vickers JC (2002) Neurofilament triplet proteins are restricted to a subset of neurons in the rat neocortex. J Chem Neuroanat 24:163-171.

Kitamura Y, Shimohama S, Kamoshima W, Ota T, Matsuoka Y, Nomura Y, Smith MA, Whitehouse PJ, Taniguchi T (1998) Alteration of proteins regulating apoptosis, Bcl-2, Bcl-x, Bax, Bak, Bad, ICH-1 and CPP32 in Alzheimer's disease. Brain Res 780:260-269.

Klunk WE, Engler H, Nordberg A, Wang Y, Blomqvist G, Holt DP, Bergstrom M, Savitcheva I, Huang GF, Estrada S, Ausen B, Debnath ML, Barletta J, Price JC, Sandell J, Lopresti BJ, Wall A, Koivisto P, Antoni G, Mathis CA, Langstrom B (2004) Imaging brain amyloid in Alzheimer's disease with Pittsburgh compound-B. Ann Neurol 55:306-319.

Klunk WE, Price JL, Mathis CA, Tsopelas ND, Lopresti BJ, Ziolko SK, Bi W, Hoge JA, Cohen AD, Ikonomovic MD, Saxton JA, Snitz BE, Pollen DA, Moonis M, Lippa CF, J.M. S, Johnson KA, Rentz DM, Fischman AJ, Aizenstein HJ, DeKosky ST

(2007) Amyloid deposition begins in the striatum of Presenilin-1 mutation carriers from two unrelated pedigrees. J Neurosci 27:6174-6184.

Knowles RB, Gomez-Isla T, Hyman BT (1998) Aβ associated neuropil changes: correlation with neuronal loss and dementia. J Neuropathol Exp Neurol 57:1122-1130.

Knowles RBW, C. Buldyrev, S.V. Cruz, L. Urbanc, B. Hasselmo, M.E. Stanley, H.E. Hyman, B.T. (1999) Plaque induced neurite abnormalities: implications for disruption of neural networks in Alzheimer's disease. Proc Natl Acad Sci U S A 96:5274-5279.

Kobayashi K, Hayashi M, Nakano H, Fukutani Y, Sasak K, Shimazaki M, Koshino Y (2002) Apoptosis of astrocytes with enhanced lysosomal activity and oligodendrocytes in white matter lesions in Alzheimer's disease. Neuropath App Neurobiol 28:238-251.

Kobayashi K, Hayashi M, Nakano H, Shimazaki M, Sugimori K, Koshino Y (2004) Correlation between astrocyte apoptosis and Alzheimer changes in gray matter lesions in Alzheimer's disease. J Alzheimer's Dis 6:623-632.

Kotilinek LA, Bacskai B, Westerman M, Kawarabayashi T, Younkin L, Hyman BT, Younkin S, Ashe KH (2002) Reversible memory loss in a mouse transgenic model of Alzheimer's disease. J Neurosci 22:6331-6335.

Kril JJ, Patel S, Harding AJ, Halliday GM (2002) Neuron loss from the hippocampus of Alzheimer's disease exceeds extracellular neurofibrillary tangle formation. Acta Neuropathol 103:370-376.

Kril JJ, Hodges J, Halliday G (2004) Relationship between hippocampal volume and CA1 neuron loss in brains of humans with and without Alzheimer's disease. Neurosci Lett 361:9-12.

Kukar T, Prescott S, Eriksen JL, Holloway V, Murphy MP, Koo EH, Golde TE, Nicolle MM (2007) Chronic administration of R-flurbiprofen attenuates learning impairments in transgenic amyloid precursor protein mice. BMC Neurosci 8:54.

Kumar-Singh S, Pirici D, McGowans E, Serneels S, Ceuterick C, Hardy J, Duff K, Dickson D, Van Broeckhoven C (2005) Dense-core plaques in Tg2576 and PSAPP

mouse models of Alzheimer's disease are centered on vessel walls. Amer J Pathol 167:527-543.

Kuo Y-M, Beach TG, Sue LI, Scott S, Layne KJ, Kokjohn TA, Kalback WM, Luehrs DC, Vishnivetskaya TA, Abramowski D, Sturchler-Pierrat C, Staufenbiel M, Weller RO, Roher AE (2001) The evolution of Aβ peptide burden in the APP23 transgenic mice: implications for Aβ deposition in Alzheimer's disease. Mol Med 7:609-618.

Kurt MA, Davies DC, Kidd M, Duff K, Howlett DR (2003) Hyperphosphorylated tau and paired helical filament-like structures in the brains of mice carrying mutant amyloid precursor protein and mutant presenilin-1 transgenes. Neurobiol Dis 14:89-97.

Kwok JBJ, Halliday GM, Brooks WS, Dolios G, Laudon H, Murayama O, Hallupp M, Badenhop RF, Vickers JC, Wang R, Naslund J, Takashima A, Gandy SE, Schofield PR (2003) Presenilin-1 mutation L271V results in altered exon 8 splicing and Alzheimer's disease with non-cored plaques and no neuritic dystrophy. J Biol Chem 278:6748-6754.

Lacor PN, Buniel MC, Furlow PW, Clemente AS, Velasco PT, Wood M, Viola KL, Klein WL (2007) Aβ oligomer-induced aberrations in synapse composition, shape, and density provide a molecular basis for loss of connectivity in Alzheimer's disease. J Neurosci 27:796-807.

Lambert MP, Stevens G, Sabo S, Barber K, Wang G, Wade W, Kraffit G, Snyder S, Holzman TF, Klein WL (1994) β/A4-evoked degeneration of differentiated SH-SY5Y human neuroblastoma cells. J Neurosci Res 39:377-385.

Lambert MP, Barlow AK, Chromy BA, Edwards C, Freed R, Liosatos M, Morgan TE, Rozovsky I, Trommer B, Viola KL, Wals P, Zhang C, Finch CE, Krafft GA, Klein WL (1998) Diffusible, nonfibrillar ligands derived from Aβ1-42 are potent central nervous system neurotoxins. Proc Natl Acad Sci U S A 95:6448-6453.

Lanz TA, Carter DB, Merchant KM (2003) Dendritic spine loss in the hippocampus of young PDAPP and Tg2576 mice and its prevention by the ApoE2 genotype. Neurobiol Dis 13:246-253.

Lassmann H, Bancher C, Breitschopf H, Wegiel J, Bobinski M, Jellinger K, Wisniewski HM (1995) Cell death in Alzheimer's disease evaluated by DNA fragmentation in situ. Acta Neuropathol 89:35-41.

Lassmann H (1996) Patterns of synaptic and nerve cell pathology in Alzheimer's disease. Behav Brain Res 78:9-14.

Lau L-F, Ahlijanian MK (2003) Role of cdk5 in the pathogenesis of Alzheimer's disease. Neuro-signals 12:209-214.

Le RL, Cruz L, Urganc B, Knowles RB, Hsiao-Ashe K, Duff K, Irizarry MC, Stanley HE, Hyman BT (2001) Plaque-induced abnormalities in neurite geometry in transgenic models of Alzheimer disease: implications for neural system disruption. J Neuropathol Exp Neurol 60:753-758.

LeBlanc A, Liu H, Goodyer C, Bergeron C, Hammonds J (1999) Caspase-6 role in apoptosis of human neurons, amyloidogenesis, and Alzheimer's Disease. J Biol Chem 274:23426-23436.

Lee J, Dickson DW, Lin W-L, Chisholm L, Corral A, Jones G, Yen S-H, Sahara N, Skipper L, Yager D, Eckman C, Hardy J, Hutton M, McGowan E (2001) Enhanced neurofibrillary degeneration in transgenic mice expressing mutant tau and APP. Science 293:1487-1491.

Lee HG, Perry G, Moreira PI, Garrett MR, Liu Q, Zhu T, Takeda A, Nunomura A, Smith MA (2005) Tau phosphorylation in Alzheimer's disease: pathogen or protector? Trends Mol Med 11:164-169.

Lee EB, Leng LZ, Zhang B, Kwong L, Trojanowski JQ, Abel T, Lee VM-Y (2006) Targeting Aβ oligomers by passive immunization with a conformation selective monoclonal antibody improves learning and memory in APP transgenic mice. J Biol Chem 281:4292-4299.

Leissring MA, Farris W, Chang AY, Walsh DM, Wu X, Sun X, Frosch MP, Selkoe DJ (2003) Enhanced proteolysis of β-amyloid in APP transgenic mice prevents plaque formation, secondary pathology, and premature death. Neuron 40:1087-1093.

Lemere CA, Blusztajn JK, Yamaguchi H, Wisniewski H, Saido TC, Selkoe DJ (1996) Sequence of deposition of heterogeneous amyloid β-peptides and APOE in Down

Syndrome: implications for initial events in amyloid plaque formation. Neurobiol Dis 3:16-32.

Lemere CA, Spooner ET, LaFrancois J, Malester B, Mori C, Leverone JF, Matsuoka Y, Taylor JW, DeMattos RB, Holtzman DM, Clements JD, Selkoe DJ, Duff K (2003) Evidence for peripheral clearance of cerebral Aβ protein following chronic, active Aβ immunization in PSAPP mice. Neurobiol Dis 14:10-18.

Leuba G, Kraftsik R, Saini K (1998) Quantitative distribution of parvalbumin, calretinin, and calbindin D-28k immunoreactive neurons in the visual cortex of normal and Alzheimer cases. Exp Neurol 152:278-291.

Levites Y, Pritam D, Price RW, Rochette MJ, Kostura LA, McGowan EM, Murphy MP, Golde TE (2006) Anti-Aβ42- and anti-Aβ40-specific mAbs attenuate amyloid deposition in an Alzheimer disease mouse model. J Clin Invest 116:193-201.

Lewen A, Li GL, Nilsson P, Olsson Y, Hillered L (1995) Traumatic brain injury in rat produces changes of β-amyloid precursor protein immunoreactivity. NeuroReport 6:357-360.

Lewis DA, Campbell MJ, Terry R, Morrison JH (1987) Laminar and regional distribution of neurofibrillary tangles and neuritic plaques in Alzheimer's disease: a quantitative study of visual and auditory cortices. J Neurosci 7:1799-1808.

Lewis J, McGowan E, Rockwood J, Melrose H, Nacharaju P, Van Slegtenhorst M, Gwinn-Hardy K, Murphy MP, Baker M, Yu X, Duff K, Hardy J, Corral A, Lin W-L, Yen S-H, Dickson DW, Davies P, Hutton M (2000) Neurofibrillary tangles, amyotrophy and progressive motor disturbance in mice expressing mutant (P301L) tau protein. Nature 25:401-405.

Lewis J, Dickson DW, Lin W-L, Chisholm L, Corral A, Jones G, Yen S-H, Sahara N, Skipper L, Yager D, Eckman C, Hardy J, Hutton M, McGowan E (2001) Enhanced neurofibrillary degeneration in transgenic mice expressing mutant tau and APP. Science 293:1487-1491.

Li GL, Farooque M, Holtz A, Olsson Y (1996) Increased expression of growth-associated protein 43 immunoreactivity in axons following compression trauma to rat spinal cord. Acta Neuropathol 92:19-26.

Li L, Cao D, Kim H, Lester R, Fukuchi K-I (2006) Simvastatin enhances learning and memory independent of amyloid load in mice. Ann Neurol 60:729-739.

Li H-L, Wang H-H, Liu S-J, Deng Y-Q, Zhang Y-J, Tian Q, Wang X-C, Chen X-Q, Yang Y, Zhang J-Y, Wang Q, Xu H, Liao F-F, Wang J-Z (2007) Phosphorylation of tau antagonizes apoptosis by stabilizing β-catenin, a mechanism involved in Alzheimer's neurodegeneration. Proc Natl Acad Sci U S A 104:3591-3596.

Lim GP, Calon F, Morihara T, Yang F, Teter B, Ubeda O, Salem NJ, Frautschy SA, Cole GM (2005) A diet enriched with the omega-3 fatty acid docosahexaenoic acid reduces amyloid burden in an aged Alzheimer mouse model. J Neurosci 25:3032-3040.

Lippa CF, Hamos JE, Pulaski-Salo D, Degennaro LJ, Drachman DA (1992) Alzheimer's disease and aging: effects on perforant pathway perikarya and synapses. Neurobiol Aging 13:405-411.

Lippa CF, Saunders AM, Smith TW, Swearer JM, Drachman DA, Ghetti B, Nee L, Pulaski-Salo D, Dickson D, Robitaille Y, Bergeron C, Crain B, Benson MD, Farlow M, Hyman BT, George-Hyslop SP, Roses AD, Pollen DA (1996) Familial and sporadic Alzheimer's disease: neuropathology cannot exclude a final common pathway. Neurology 46:406-412.

Livingston G, Katona C (2004) The place of memantine in the treatment of Alzheimer's disease: a number needed to treat analysis. Int J Geriatr Psychiatry 19:919-925.

Lombardo JA, Sterns EA, McLellan ME, Kajdasz ST, Hickey GA, Bacskai BJ, Hyman BT (2003) Amyloid-β antibody treatment leads to rapid normalization of plaque-induced neuritic alterations. J Neurosci 23:10883-10879.

Loo DT, Copani A, Pike CJ, Whittemore ER, Walencewicz AJ, Cotman CW (1993) Apoptosis is induced by β-amyloid in cultured central nervous system neurons. Proc Natl Acad Sci U S A 90:7951-7955.

Lord A, Kalimo H, Eckman C, Zhang X-Q, Lannfelt L, Nilsson LNG (2006) The arctic Alzheimer mutation facilitates early intraneuronal Aβ aggregation and senile plaque formation in transgenic mice. Neurobiol Aging 27:67-77.

Lorenzo A, Yanker BA (1994) β-amyloid neurotoxicity requires fibril formation and is inhibited by Congo red. Proc Natl Acad Sci U S A 91:12243-12247.

Lovasic L, Bauschke H, Janus C (2005) Working memory impairment in a transgenic amyloid precursor protein TgCRND8 mouse model of Alzheimer's disease. Genes Brain Behav 4:197-208.

Lu DC, Rabizadeh S, Chandra S, Shayya RF, Ellerby LM, Ye X, Salvesen GS, Koo EH, Bredesen DE (2000) A second cytotoxic proteolytic peptide derived from amyloid β-protein precursor. Nat Med 6:397-404.

Lu DC, Shaked GM, Masliah E, Bredesen DE, Koo EH (2003) Amyloid β protein toxicity mediated by the formation of amyloid-β protein precursor complexes. Ann Neurol 54:781-789.

Lucassen PJ, Chung WC, Kamphorst W, Swaab DF (1997) DNA damage distribution in the human brain as shown by in situ end labeling; area-specific differences in aging and Alzheimer disease in the absence of apoptotic morphology. J Neuropathol Exp Neurol 56:887-900.

Luchsinger JA, Tang MX, Shea S, Mayeux R (2002) Caloric intake and the risk of Alzheimer's disease. Arch Neurol 59:1258-1263.

Luchsinger JA, Tang MX, Shea S, Mayeux R (2003) Antioxidant vitamin intake and risk of Alzheimer's disease. Arch Neurol 60:203-208.

Luchsinger JA, Nobles JM, Scarmeas N (2007) Diet and Alzheimer's disease. Curr Neurol Neurosci Rep 7:366-372.

Lue LF, Brachova L, Civin WH, Rogers J (1996) Inflammation, Abeta deposition, and neurofibrillary tangle formation as correlates of Alzheimer's disease neurodegeneration. J Neuropathol Exp Neurol 55:1083-1088.

Lund RJ, Ylikoski EK, Aittokallio T, Nevalainen O, Lahesmaa R (2003) Kinetics and STAT4- or STAT6-mediated regulation of genes involved in lymphocyte polarization to Th1 and Th2 cells. Eur J Immunol 33:1105-1116.

Lyketsos CG, Breitner JC, Green RC, Martin BK, Meinert C, Piantadosi S, Sabbagh M (2007) Naproxen and celecobix do not prevent AD in early results from a randomized controlled trial. Neurology 68:1800-1808.

Ma H, Lesne S, Kotilinek L, Steidl-Nichols JV, Sherman M, Younkin L, Younkin S, Forster C, Sergeant N, Delacourte A, Vassar R, Citron M, Kofuji P, Boland LM, Ashe KH (2007a) Involvement of β-site APP cleaving enzyme 1 (BACE1) in

amyloid precursor protein-mediated enhancement of memory and activity-dependent synaptic plasticity. Proc Natl Acad Sci U S A 104:8167-8172.

Ma Q, Cornelli U, Hanin I, Jeske WP, Linhardt RJ, Walenga JM, Fareed J, Lee JM (2007b) Heparin oligosaccharides as potential therapeutic agents in senile dementia. Curr Pharm Des 13:1607-1616.

MacGibbon GA, Lawlor PA, Sirimanne SE, Walton MR, Connor B, Young D, Williams C, Gluckman P, Faull RLM, Hughes P, Dragunow M (1997) Bax expression in mammalian neurons undergoing apoptosis, and in Alzheimer's disease hippocampus. Brain Res 750:223-234.

Maier M, Seabrook TJ, Lazo ND, Jiang L, Das P, Janus C, Lemere CA (2006) Short amyloid-beta (Abeta) immunogens reduce cerebral Abeta load and learning deficits in an Alzheimer's disease mouse model in the absence of an Abeta-specific cellular immune response. J Neurosci 26:4717-4728.

Manczak M, Jung Y, Park BS, Partovi D, Reddy PH (2005) Time-course of mitochondrial gene expressions in mice brains: implications for mitochondrial dysfunction, oxidative damage, and cytochrome c in aging. J Neurochem 92:494-504.

Manczak M, Anekonda TS, Henson E, Park BS, Quinn J, Reddy PH (2006) Mitochondria are a direct site of Aβ accumulation in Alzheimer's disease neurons: implications for free radical generation and oxidative damage in disease progression. Human Molecular Genetics 15:1437-1449.

Mandelkow EM, Drewes G, Biernalt J, Gustke N, Van Lint J, Vandenheeds JR, Mandelkow E (1992) Glycogen synthase kinase-3 and the Alzheimer-like state of microtubule-associated protein tau. FEBS Lett 314:315-321.

Mann DMA, Takeuchi A, Sato S, Cairns NJ, Lantos PL, Rossor MN, Hailtai M, Kalimo H, Iwatsubo T (2001) Cases of Alzheimer's disease due to deletion of exon 9 of the presenilin-1 gene show an unusual but characteristic β-amyloid pathology known as 'cotton wool' plaques. Neuropath App Neurobiol 27:189-196.

Marambaud P, Zhao H, Davies P (2005) Resveratrol promotes clearance of Alzheimer's disease amyloid-β peptides. J Biol Chem 280:37377-37382.

Maret W (1995) Metallothionein/disulfide interactions, oxidative stress, and the mobilization of cellular zinc. Neurochem Int 27:111-117.

Markesbery WR, Carney JM (1999) Oxidative alterations in Alzheimer's disease. Brain Pathol 9:133-146.

Marmarou CR, Walker SA, Davis CL, Povlishock JT (2005) Quantitative analysis of the relationship between intra-axonal neurofilament compaction and impaired axonal transport following diffuse traumatic brain injury. J Neurotrauma 22:1066-1080.

Marques CA, Keit U, Bonert A, Steiner B, Haass C, Muller WE, Eckert A (2003) Neurotoxic mechanisms caused by the Alzheimer's disease-linked Swedish amyloid precursor protein mutation. J Biol Chem 278:28294-28392.

Marszalek JR, Lodish HF (2005) Docosahexaenioc acid, fatty acid-interacting proteins, and neuronal function: breastmilk and fish are good for you. Ann Rev Cell Dev Biol 21:633-657.

Martin LJ, Pardo CA, Cork LC, Price DL (1994) Synaptic pathology and glial response to neuronal injury precede the formation of senile plaques and amyloid deposits in the aging cerebral cortex. Amer J Pathol 145:1358-1381.

Martin LJ, Liu Z (2002) Injury-induced spinal motor neuron apoptosis is preceded by DNA single-strand breaks and is p53 and Bax-dependent. J Neurobiol 50:181-197.

Masliah E, Mallory M, Hansen L, Alford M, Albright T, DeTeresa R, Terry R, Baudier J, Saitoh T (1991) Patterns of aberrant sprouting in Alzheimer's disease. Neuron 6:729-739.

Masliah E, Mallory M, Ge N, Saitoh T (1992a) Amyloid precursor protein is localized in growing neurites of neonatal rat brain. Brain Res 593:323-328.

Masliah E, Mallory M, Hansen L, Alford M, DeTeresa R, Terry R, Baudier J, Saitoh T (1992b) Localization of amyloid precursor protein in GAP43-immunoreactive aberrant sprouting neurites in Alzheimer's disease. Brain Res 574:312-316.

Masliah E, Mallory M, Hansen L, Alford M, DeTeresa R, Terry R (1993) An antibody against phosphorylated neurofilaments identifies a subset of damaged association axons in Alzheimer's disease. Amer J Pathol 142:871-881.

Masliah E, Mallory M, Alford M, Mucke L (1994) Abnormal synaptic regeneration in hAPP transgenic and APOE-knockout mice. Neurobiol Aging 15:S11.

Masliah E, Mallory M, Ge N, Alford M, Veinbergs I, Roses AD (1995) Neurodegeneration in the central nervous system of apoE-deficient mice. Exp Neurol 136:107-122.

Masliah E, Mallory M, Veinbergs I, Miller A, Samuel W (1996a) Alterations in apolipoprotein E expression during aging and neurodegeneration. Prog Neurobiol 50:493-503.

Masliah E, Sisk A, Mallory M, Mucke L, Schenk D, Games D (1996b) Comparison of neurodegenerative pathology in transgenic mice overexpressing human β-amyloid precursor protein and Alzheimer's disease. Neurosci 16:5795-5811.

Masliah E, Mallory M, Alford M, Tanka S, Hansen LA (1998) Caspase dependent DNA fragmentation might be associated with excitotoxicity in Alzheimer's disease. J Neuropathol Exp Neurol 57:1041-1052.

Masliah E, Sisk A, Mallory M, Games D (2001) Neurofibrillary pathology in transgenic mice overexpressing V717F β-Amyloid precursor protein. J Neuropathol Exp Neurol 60:357-368.

Mastrangelo P, Mathews PM, Chishti MA, Schmidt SD, Gu Y, Yang J, Mazzella MJ, Coomaraswamy J, Horne P, Strome B, Pelly H, Levesque G, Ebeling C, Jiang Y, Nixon RA, Rozmahel R, Fraser PE, St George-Hyslop P, Carlson GA, Westaway D (2005) Dissociated phenotypes in presenilin transgenic mice define functionally distinct gamma-secretases. Proc Natl Acad Sci U S A 102:8972-8977.

Matsumoto Y, Yanase D, Noguchi-Shinohara M, Ono K, Yoshita M, Yamada M (2007) Blood-brain barrier permeability correlates with medial temporal lobe atrophy but not with amyloid-beta protein transport across the blood-brain barrier in Alzheimer's disease. Dement Geriatr Cogn Disord 23:241-245.

Matsuyama S, Teraoka R, Mori H, Tomiyama T (2007) Inverse correlation between amyloid precursor protein and synaptic plasticity in transgenic mice. NeuroReport 18:1083-1087.

Maynard CJ, Cappai R, Volitakis I, Cherny RA, White AR, Beyreuther K, Masters CL, Bush AI, Li QX (2002) Overexpression of Alzheimer's disease amyloid-beta opposes the age-dependent elevations of brain copper and iron. J Biol Chem 277:44670-44676.

McConlogue L, Buttini M, Anderson JP, Brigham EF, Chen KS, Freedman SB, Games D, Jonhson-Wood K, Lee M, Zeller M, Liu W, Motter R, Sinha S (2007) Partial reduction of BACE1 has dramatic effects on Alzheimer's plaques and synaptic pathology in APP transgenic mice. J Biol Chem 282:26326-26334.

McGowan E, Sanders S, Iwatsubo T, Takeuchi A, Saido T, Zehr C, Yu X, Uljon S, Wang R, Mann D, Dickson D, Duff K (1999) Amyloid phenotype characterization of transgenic mice overexpressing both mutant amyloid precursor protein and mutant presenilin 1 transgenes. Neurobiol Dis 6:231-244.

McGowan E, Eriksen J, Hutton M (2006) A decade of modeling Alzheimer's disease in transgenic mice. Trends Genetics 22:281-289.

McKee AC, Kosik KS, Kowall NW (1991) Neuritic pathology and dementia in Alzheimer's disease. Ann Neurol 30:156-165.

McLaurin J, Kierstead ME, Brown ME, Hawkes CA, Lambermon MHL, Phinney AL, Darabie AA, Cousins JE, French JE, Lan MF, Chen F, Wong SSN, Mount HTJ, Fraser PE, Westaway D, St George-Hyslop P (2006) Cyclohexanehexol inhibitors of Aβ aggregation prevent and reverse Alzheimer phenotype in a mouse model. Nat Med 7:801-808.

McShea A, Harris PLF, Webster KR, Wahl AF, Smith A (1997) Abnormal expression of the cell cycle regulators p16 and cdk4 in Alzheimer's disease. Amer J Pathol 150:1933-1939.

Meller D, Eysel UT, Schmidt-Kastner R (1994) Transient immunohistochemical labelling of rat retinal axons during Wallerian degeneration by a monoclonal antibody to neurofilaments. Brain Res 648:162-166.

Mesulam MM (1999) Neuroplasticity failure in Alzheimer's disease: bridging the gap between plaques and tangles. Neuron 24:521-529.

Metsaars WP, Hauw J-J, Van Welsem ME, Duyckaerts C (2003) A grading system of Alzheimer disease lesions in neocortical areas. Neurobiol Aging 24:563-572.

Mi K, Johnson GV (2006) The role of tau phosphorylation in the pathogenesis of Alzheimer's disease. Curr Alzheimer Res 3:449-463.

Michaelis ML, Ranciat N, Chen Y, Bechtel M, Ragan R, Hepperle M, Liu Y, George G (1998) Protection against β-amyloid toxicity in primary neurons by Paclitaxel (Taxol). J Neurochem 70:1623-1627.

Michaelis ML, Chen Y, Hill S, Reiff E, George G, Rice A, Audus K (2002) Amyloid peptide toxicity and microtubule-stabilizing drugs. J Mol Neurosci 19:101-105.

Michaelis ML (2006) Ongoing in vitro studies with cytoskeletal drugs in tau transgenic mice. Curr Alzheimer Res 3:215-219.

Middei S, Daniele S, Caprioli A, Ghirardi O, Ammassari-Teule M (2006) Progressive cognitive decline in a transgenic mouse model of Alzheimer's disease overexpressing mutant hAPPswe. Genes Brain Behav 5:249-256.

Miller CC, Ackerley S, Brownlees J, Grierson AJ, Jacobsen NJ, Thornhill P (2002) Axonal transport of neurofilaments in normal and disease states. Cell Mol Life Sci 59:323-330.

Millet P, Lages CS, Haik S, Nowak E, Allemand I, Granotier C, Boussin FD (2005) Amyloid-β peptide triggers Fas-independent apoptosis and differentiation of neural progenitor cells. Neurobiol Dis 19:57-67.

Mintun MA, Larossa GN, Sheline YI, Dence CS, Lee SY, Mach RH, Klunk WE, Mathis CA, DeKosky ST, Morris JC (2006) [11C]PIB in a nondemented population: potential antecedent marker of Alzheimer disease. Neurology 67:446-452.

Mirra SS, Heyman A, McKeel D, Sumi SM, Brownlee LM, Vogel FS, van Belle G, Berg L (1991) The consortium to establish a registry for Alzheimer's disease (CERAD) Part II Standardization of the neuropathological assessment of Alzheimer's disease. Neurology 41:479-486.

Moechars D, Dewachter I, Lorent K, Reversé D, Baekelandt V, Naidu A, Tesseur I, Spittaels K, Haute CV, Checler F, Godaux E, Cordell B, Van Leuven F (1999) Early phenotypic changes in transgenic mice that overexpress different mutants of amyloid precursor protein in brain. J Biol Chem 274:6483-6492.

Molnár Z, Cheung AFP (2006) Towards the classification of subpopulations of layer V pyramidal projection neurons. Neurosci Res 55:105-115.

Moolman DL, Vitolo OV, Vonsattel J-PG, Shelanksi ML (2004) Dendrite and dendritic spine alterations in Alzheimer models. J Neurocytol 33:377-387.

Morgan D, Diamond DM, Gottschall PE, Ugens KE, Kickey C, Hardy J, Duff K, Jantzen P, DiCarlo G, Wilcock D, Conner K, Hatcher J, Hope C, Gordon M, Arendash GW (2001) Aβ peptide vaccination prevents memory loss in an animal model of Alzheimer's disease. Nature 408:982-985.

Morishima Y, Gotoh Y, Zieg J, Barrett T, Takano H, Flavell R, Davis RJ, Shirasaki Y, Greenberg ME (2001) β-amyloid induces neuronal apoptosis via a mechanism that involves the c-Jun N-terminal kinase pathway an the induction of Fas ligand. J Neurosci 21:7551-7560.

Morris JC, Storandt M, McKeel DW, Rubin EH, Price JL, Grant EA, Berg L (1996) Cerebral amyloid deposition and diffuse plaques in "normal" aging: Evidence from presymptomatic and very mild Alzheimer's disease. Neurology 46:707-719.

Morris MC, Evans DA, Bienias JL, Tangney CC, Bennett DA, Aggarwal NT, Scherr PA (2002) Dietary intake of antioxidant nutrients and the risk of Alzheimer's disease in a biracial community study. JAMA 287:3230-3237.

Morris MC, Evans DA, Tangney CC, Bienias JL, Wilson RS, Aggarwal NT, Scherr PA (2005) Relation of the tocopherol forms to incident Alzheimer's disease and to cognitive change. Am J Clin Nutr 81:508-514.

Morrison JH, Lewis DA, Campbell MJ (1987) Distributions of neurofibrillary tangles and nonphosphorylated neurofilament protein-immunoreactive neurons in cerebral cortex: implications for loss of corticocortical circuits in Alzheimer's disease. In: Banbury Report 27: Molecular Neuropathology of Aging (Davies P, Finch C, eds), pp 109-124. New York: Cold Spring Harbor Laboratory.

Morsch R, Simon W, Coleman PD (1999) Neurons may live for decades with neurofibrillary tangles. J Neuropathol Exp Neurol 58:188-197.

Motoi Y, Itaya M, Mori H, Mizuno Y, Iwasaki T, Hattori H, Haga S, Ikeda K (2004) Apolipoprotein E receptor 2 is involved in neuritic plaque formation in APP sw mice. Neurosci Letters 368:144-147.

Movsesyan VA, Stoica BA, A.I. F (2004) MGLuR5 activation reduces β-amyloid-induced cell death in primary neuronal cultures and attenuates translocation of cytochrome c and apoptosis-inducing factor. J Neurochem 89:1528-1536.

Mucke L, Masliah E, Yu G-Q, Mallory M, Rockenstein EM, Tatsuno G, Hu K, Kholodenko D, Johnson-Wood K, McConlogue L (2000) High-level neuronal expression of Aβ1-42 in wild-type human amyloid protein precursor transgenic mice: synaptotoxicity without plaque formation. J Neurosci 20:4050-4058.

Muzio M, Stockwell BR, Stennicke HR, Salvesen GS, Dixit VM (1998) An induced proximity model for caspase-8 activation. J Biol Chem 273:2926-2930.

Nagy ZS, Esiri MM (1997) Apoptosis-related protein expression in the hippocampus in Alzheimer's disease. Neurobiol Aging 18:565-571.

Nakamura Y, Hasimoto R, Kashiwagi Y, Miyamae Y, Shinosaki K, Nishikawa T, Hattori H, Kudo T, Takeda M (1997) Abnormal distribution of neurofilament L in neurons with Alzheimer's disease. Neurosci Lett 225:201-204.

Nangano S, Satoh M, Sumi H, Fujimura H, Tohyama C, Yanagihara T, Sakoda S (2001) Reduction of metallothioneins promotes the disease expression of familial amyotrophic lateral sclerosis mice in a dose-dependent manner. Eur J Neurosci 13:1363-1370.

Ness BK, Boggs LN, Hepburn DL, Gitter B, Long GG, May PC, Piroozi KS, Schafer KA, Yang Z (2004) Reduced β-amyloid burden, increased C-99 concentrations and evaluation of neuropathology in the brains of PDAPP mice given Ly450139 dihydrate daily by gavage for 5 months. Neurobiol Aging 25:S238-S239.

Nicoll JAR, Wilkinson D, Holmes C, Steart P, Markham H, Weller RO (2003) Neuropathology of human Alzheimer disease after immunization with amyloid-β peptide: a case report. Nat Med 9:448-452.

Nicoll JA, Barton E, Boche D, Neal JW, Ferrer I, Thompson P, Vlachouli C, Wilkinson D, Bayer A, Games D, Seubert P, Schenk D, Holmes C (2006) Aβ species removal after Aβ42 immunization. J Neuropathol Exp Neurol 65:1040-1048.

Noble W, Olm V, Takata K, Casey OM, Meyerson J, Gaynor K, LaFrancois J, Wang L, Kondo T, Davies P, Burns M, Veeranna, Nixon R, Dickson D, Matsuoka Y, Ahlijanian M, Lau L-F, Duff K (2003) Cdk5 is a key factor in tau aggregation and tangle formation in vivo. Neuron 38:555-565.

Noda-Saita K, Terai K, Iwai A, Tsukamoto M, Shitaka Y, Kawabata S, Okada M, Yamaguchi T (2004) Exclusive association and simultaneous appearance of

congophilic plaques and AT8-positive dystrophic neurites in Tg2576 mice suggest a mechanism of senile plaque formation and progression of neuritic dystrophy in Alzheimer's disease. Acta Neuropathol 108:435-442.

Nunomura A, Chiba S (2000) Avoidance of apoptosis in Alzheimer's disease. J Alzheimer's Dis 2:59-60.

Oakley H, Cole S, Logan S, Maus E, Shao P, Craft J, Guillozet-Bongaarts A, Ohno M, Disterhoft J, Van Eldik L, Berry R, Vassar R (2006) Intraneuronal β-amyloid aggregates, neurodegeneration, and neuron loss in transgenic mice with five familial Alzheimer's disease mutations: potential factors in amyloid plaque formation. J Neurosci 26:10129-10140.

Oddo S, Caccamo A, Shepherd JD, Murphy MP, Golde TE, Kayed R, Metherate R, Mattson MP, Akbari Y, LaFerla FM (2003a) Triple-transgenic model of Alzheimer's disease with plaques and tangles: intracellular Aβ and synaptic dysfunction. Neuron 39:409-421.

Oddo S, Caccamo A, Kitazawa M, Tseng BP, LaFerla FM (2003b) Amyloid deposition precedes tangle formation in a triple transgenic model of Alzheimer's disease. Neurobiol Aging 24:1063-1070.

Oddo S, Billings L, Kesslak JP, Cribbs DH, LaFerla FM (2004) Aβ immunotherapy leads to clearance of early, but not late, hyperphosphorylated tau aggregates via the proteasome. Neuron 43:321-332.

Oddo S, Vasilevko V, Caccamo A, Kitazawa M, Cribbs DH, LaFerla FM (2006) Reduction of soluble Aβ and tau, but not soluble Aβ alone, ameliorates cognitive decline in transgenic mice with plaques and tangles. J Biol Chem 281:39413-39423.

Oddo S, Caccamo A, Cheng D, Jouleh B, Torp R, LaFerla FM (2007) Genetically augmenting tau levels does not modulate the onset or progression of Aβ pathology in transgenic mice. J Neurochem 102:1053-1063.

Ogawa O, Lee H-G, Zhu X, Raina A, Harris PLR, Castellani RJ, Perry G, Smith MA (2003) Increased p27, an essential component of cell cycle control, in Alzheimer's disease. Aging Cell 2:105-110.

Ohyagi Y, Asahara H, Chui D-H, Tsuruta Y, Sakae N, Miyoshi K, Yamada T, Kikuchi H, Taniwaki T, Murai H, Ikezoe K, Furuya H, Kawarabayashi T, Shoji M, Checler

F, Iwaki T, Makifuchi T, Takeda K, Kira J-I, Tabira T (2005) Intracellular Aβ42 activates p53 promoter: a pathway to neurodegeneration in Alzheimer's disease. FASEB J 19:255-257.

Okura Y, Miyakoshi A, Kohyama K, Park I, Staufenbiel M, Matsumoto Y (2006) Nonviral Aβ DNA vaccine therapy against Alzheimer's disease: Long-term effects and safety. Proc Natl Acad Sci U S A 103:9619-9624.

Ono K, Naiki H, Yamada M (2006) The development of preventives and therapeutics for Alzheimer's disease that inhibit the formation of β-amyloid fibrils (fAβ), as well as destabilize preformed fAβ. Curr Pharm Design 12:4357-4375.

Onorato M, Molvihill P, Connolly J, Galloway P, Whitehouse P, Perry G (1989) Alteration of neuritic cytoarchitecture in Alzheimer disease. Prog Clin Biol Res 317:781-789.

Opazo C, Huang X, Cherny RA, Moir RD, Roher AE, White AR, Cappai R, Masters CL, Tanzi RE, Inestrosa NC, Bush AI (2002) Metalloenzyme-like activity of Alzheimer's disease beta-amyloid. Cu-dependent catalytic conversion of dopamine, cholesterol, and biological reducing agents to neurotoxic H(2)O(2). J Biol Chem 277:40302-40308.

Orgogozo J-M, Gilman S, Dartigues J-F, Laurent B, Puel M, Kirby LC, Jouanny P, Dubois B, Eisner L, Flitman S, Michel BF, Boada M, Frank A, Hock C (2003) Subacute meningoencephalitis in a subset of patients with AD after Aβ42 immunization. Neurology 61:46-54.

Otsuka N, Tomonaga M, Ikeda K (1991) Rapid appearance of β-amyloid precursor protein immunoreactivity in damaged axons and reactive cells in rat brain following needle stab injury. Brain Res 568:335-338.

Overmyer M, Draszpulski M, Helisalmi S, Soininen H, Alafuzoff I (2000) DNA fragmentation, gliosis and histological hallmarks of Alzheimer's disease. Acta Neuropathol 100:681-687.

Page AB, Owen CR, Kumar R, Miller JM, Rafols JA, White BC, DeGracia DJ, Krause GS (2003) Persistent elF2a(P) is colocalized with cytoplasmic cytochrome c in vulnerable hippocampal neurons after 4 hours of reperfusion following 10-minute complete brain ischemia. Acta Neuropathol 106:8-16.

Panisset M, Gauthier S, Moessler H, Windisch M, The Cerebrolysin Study Group (2002) Cerebrolysin in Alzheimer's disease: a randomized, double-blind, placebo-controlled trial with a neurotrophic agent. J Neural Transm 109:1089-1104.

Paradis E, Douillard H, Koutroumanis M, Goodyer C, LeBlanc A (1996) Amyloid β peptide of Alzheimer's disease downregulates Bcl-2 and upregulates Bax expression in human neurons. J Neurosci 16:7533-7539.

Paradisi S, Sacchetti B, Balduzzi M, Gaudi S, Malchiodi-albedi F (2004) Astrocyte modulation of in vitro β-amyloid neurotoxicity. Glia 46:252-260.

Park KHJ, Hallows JL, Chakrabarty P, Davies P, Vincent I (2007) Conditional neuronal simian virus 40 T antigen expression induces AD-like tau and amyloid pathology in mice. J Neurosci 27:2969-2978.

Parnetti L, Senin U, Mecocci P (1997) Cognitive enhancement therapy for Alzheimer's disease: The way forward. Drugs 53:752-768.

Patton RL, Kalback WM, Esh CL, Kokjohn TA, Van Vickle GD, Luehrs DC, Kuo Y-M, Lopez J, Brune D, Ferrer I, Masliah E, Newel AJ, Beach TG, Castano EM, Roher AE (2006) Amyloid-β peptide remnants in AN-1792-immunized Alzheimer's disease patients. Amer J Pathol 169:1048-1063.

Pearson RCA, Esiri MM, Hiorns RW, Wilcock GK, Powell TPS (1985) Anatomical correlates of the distribution of the pathological changes in the neocortex in Alzheimer's disease. Proc Natl Acad Sci U S A 82:4531-4534.

Pedersen WA, McMillan PJ, Kulstad JJ, Leverenz JB, Craft S, Haynatzki GR (2006) Rosiglitazone attenuates learning and memory deficits in Tg2576 Alzheimer mice. Exp Neurol 199:265-273.

Penkowa M, Carrasco J, Giralt M, Moos T, Hidalgo J (1999) CNS wound healing is severely depressed in metallothionein I- and II-deficient mice. J Neurosci 19:2535-2545.

Penkowa M, Hidalgo J (2003) Treatment with MT prevents demyelination and axonal damage and increases tissue repair during experimental autoimmune encephalomyelitis. J Neurosci Res 72:574-586.

Penkowa M, Espejo C, Martinez-Caceres EM, Montalban X, Hidalgo J (2003) Increased demyelination and axonal damage in metallothionein I+II deficient mice during experimental autoimmune encephalomyelitis. Cell Mol Life Sci 60:185-197.

Perez M, Ribe E, Rübio A, Lim F, Moran MA, Gomez Ramos P, Ferrer I, Isla MTG, Avila J (2005) Characterization of a double (amyloid precursor protein-tau) transgenic: tau phosphorylation and aggregation. Neurosci 130:339-347.

Perry G, Nunomura A, Lucassen P, Lassmann H, Smith MA (1998) Apoptosis in Alzheimer's disease. Science 282:1268-1269.

Peters A, Sethares C (1996) Myelinated axons and pyramidal cell modules in monkey primary visual cortex. J Comp Neurol 365:323-355.

Peters A, Cifuentes M, Sethares C (1997) The organization of pyramidal cells in area 18 of the rhesus monkey. Cereb Cortex 7:405-421.

Phinney AL, Deller T, Stalder M, Calhoun ME, Frotscher M, Sommer B, Staufenbiel M, Jucker M (1999) Cerebral amyloid induces aberrant axonal sprouting and ectopic terminal formation in amyloid precursor protein transgenic mice. J Neurosci 19:8552-8559.

Phinney AL, Horne P, Yang J, Janus C, Bergeron C, Westaway D (2003) Mouse models of Alzheimer's disease: The long and filamentous road. Neurolog Res 25:590-600.

Piccini A, Russo C, Gliozzi A, Relini A, Vitali A, Borghi R, Giliberto L, Armirotti A, D'Arrigo C, Bachi A, Catteneo A, Canale C, Torrassa S, Saido TC, Markesbery W, Gambetti P, Tabaton M (2005) β-amyloid is different in normal aging and in Alzheimer disease. J Biol Chem 280:34186-34192.

Pike CJ, Cummings BJ, Cotman CW (1992) β-amyloid induces neuritic dystrophy in vitro: similarities with Alzheimer pathology. NeuroReport 3:769-772.

Pike CJ, Burdick D, Walencewicz AJ, Glabe CG, Cotman CW (1993) Neurodegeneration induced by β-amyloid peptides in vitro: the role of peptide assembly state. J Neurosci 13:1676-1687.

Pike CJ, Walencewicz-Wasserman AJ, Kosmoski J, Cribbs DH, Glabe CG, Cotman CW (1995) Structure-activity analyses of β-amyloid peptides: contributions of the Aβ25-35 region to aggregation and neurotoxicity. J Neurochem 64:253-265.

Pillot T, Drouet B, Queille S, Labeur C, Vandekerckhove J, Rosseneu M, Pincon-Raymond M, Chambaz J (1999) The nonfibrillar amyloid β-peptide induces apoptotic neuronal cell death: involvement of its c-terminal fusogenic domain. J Neurochem 73:1626-1634.

Poirier J (1994) Apolipoprotien E in animal models of CNS injury and in Alzheimer's disease. Trends Neurosci 17:525-530.

Pollack M, Phaneuf S, Dirks A, Leeuwenburgh C (2002) The role of apoptosis in the normal aging brain, skeletal muscle, and heart. Ann N Y Acad Sci 959:93-107.

Pompl PN, Yemul S, Xiang Z, Ho L, Harutunian V, Purohit D, Mohs R, Pasinetti GM (2003) Caspase gene expression in the brain as a function of the clinical progression of Alzheimer disease. Arch Neurol 60:360-376.

Prasad CV, Zheng M, Vig S, Bergstrom C, Smith DW, Gao O, Yeolas S, Polson CT, Cosa JA, Guss VL, Loo A, Wang J, Sleczka BG, Dangler C, Robertson BJ, Hendrick JP, Roberts SB, Barten DM (2007) Discovery of (S)-2-((S)-2-(3,5-difluorophenyl)-2-hydroxyacetamido)-N-((S,Z)-3-methyl-4-oxo-4,5-dihydro-3H-benzo[d][1,2]diazepin-5-yl)propanamide (BMS-433796): a gamma-secretase inhibitor with Abeta lowering activity in a transgenic mouse model of Alzheimer's disease. Bioorg Med Chem Lett 17:4006-4011.

Pravdenkova SV, Basnakian AG, James SJ, Anderson BJ (1996) DNA fragmentation and nuclear endonuclease activity in rat brain after severe closed head injury. Brain Res 729:151-155.

Price JL, Morris JC (1999) Tangles and plaques in nondemented aging and "preclinical" Alzheimer's disease. Ann Neurol 45:358-368.

Puttaparthi K, Gitomer WL, Krishnan U, Son M, Rajendran B, Elliot JL (2002) Disease progression in a transgenic model of familial amyotrophic lateral sclerosis is dependent on both neuronal and non-neuronal zinc binding proteins. J Neurosci 22:8790-8796.

Qu B, Rosenberg RN, Li L, Boyer PJ, Johnston SA (2004) Gene vaccination to bias the immune response to amyloid-β peptide as therapy for Alzheimer disease. Arch Neurol 61:1859-1864.

Rabano A, Jimenez-Huete A, Acevedo B, Calero M, Chiso J, Valdes I, Gavilondo J, Frangione B, Mendez E (2005) Diversity of senile plaques in Alzheimer's disease as revealed by a new monoclonal antibody that recognizes an internal sequence of the Aβ peptide. Curr Alzheimer Res 2:409-417.

Raina AK, Hochman A, Zhu X, Rottkamp CA, Nunomura A, Siedlak SL, Boux H, Castellani RJ, Perry G, Smith MA (2001) Abortive apoptosis in Alzheimer's disease. Acta Neuropathol 101:305-310.

Raina AK, Hochman A, Ickes II H, Zhu X, Ogawa O, Cash AD, Shimohama S, Perry G, Smith MA (2003) Apoptotic promoters and inhibitors in Alzheimer's disease: Who wins out? Prog Neuro-Psychopharm Bio Psych 27:251-254.

Ramirez G, Toro R, Dobeli H, Bernhardi RV (2005) Protection of rat primary hippocampal cultures from Aβ cytotoxicity by pro-inflammatory molecules is mediated by astrocytes. Neurobiol Dis 19:243-254.

Ravaglia G, Forti P, Maioli F, Bastagli L, Montesi F, Pisacane N, Chiappelli M, Licastro F, Patterson C (2007) Endogenouse sex hormones as risk factors for dementia in elderly men and women. J Gerontol A Biol Sci Med Sci 62:1035-1041.

Reid PC, Urano Y, Kodama T, Hamakubo T (2007) Alzheimer's disease: cholesterol, membrane rafts, isoprenoids and statins. J Cell Mol Med 11:383-392.

Reilly JF, Games D, Rydel RE, Freedman S, Schenk D, Young WG, Morrison JH, Bloom FE (2003) Amyloid deposition in the hippocampus and entorhinal cortex: quantitative analysis of a transgenic mouse model. Proc Natl Acad Sci U S A 100:4837-4842.

Rezai-Zadeh K, Shytle D, Sun N, Mori T, Hou H, Jeanniton D, Ehrhart J, Townsend K, Zeng J, Morgan D, Hardy J, Town T, Tan J (2005) Green tea epigallocatechin-3-gallate (EGCG) modulates amyloid precursor protein cleavage and reduces cerebral amyloidosis in Alzheimer transgenic mice. J Neurosci 25:8807-8814.

Ribe EM, Perez M, Puig B, Gich I, Lim F, Cuadrado M, Sesma T, Catena S, Sanchez B, Nieto M, Gomez-Ramos P, Moran MA, Cabodevila F, Samaranch L, Ortiz L, Perez A, Ferrer I, Avila J, Gomez-Isla T (2005) Accelerated amyloid deposition, neurofibrillary degeneration and neuronal loss in double mutant APP/tau transgenic mice. Neurobiol Dis 20:814-822.

Richards JG, Higgins GA, Ouagazzal A-M, Ozmen L, Kew JNC, Bohrmann B, Malherve P, Brockhaus M, Loetscher H, Czech C, Huber G, Bluethmann H, Jacobsen H, Kemp JA (2003) PS2APP transgenic mice, coexpressing hPS2mut and hAPPswe, show age-related cognitive deficits associated with discrete brain amyloid deposition and inflammation. J Neurosci 23:8989-9003.

Riddell DR, Zhou H, Comery TA, Kouranova E, Lo CF, Warwick HK, Ring RH, Kirksey Y, Aschmies S, Xu J, Kubek K, Hirst WD, Gonzales C, Chen Y, Murphy E, Leonard S, Vasylyev D, Oganesian A, Martone RL, Pangalos MN, Reinhart PH, Jacobsen JS (2007) The LXR agonist TO901317 selectively lowers hippocampal Aβ42 and improves memory in the Tg2576 mouse model of Alzheimer's disease. Mol Cell Neurosci 34:621-628.

Ring S, Weyer SW, Kilian SB, Waldron E, Pietrzik CU, Filippov MA, Herms J, Buchholz C, Eckman C, Korte M, Wolfer DP, Muller UC (2007) The secreted β-amyloid precursor protein ectodomain APPsα is sufficient to rescue anatomical, behavioral, and electrophysiological abnormalities of APP-deficient mice. J Neurosci 27:7817-7826.

Risner ME, Saunders AM, Altman JFB, Ormandy GC, Craft S, Foley IM, Zvartau-Hind ME, Hosford DA, Roses AD (2006) Efficacy of rosiglitazone in a genetically defined population with mild-to-moderate Alzheimer's disease. Pharmacogenomics J 6:246-254.

Rissman RA, Poon WW, Blurton-Jones M, Oddo S, Torp R, Vitek MP, LaFerla FM, Rohn TT, Cotman CW (2004) Caspase-cleavage of tau is an early event in Alzheimer disease tangle pathology. J Clin Invest 114:121-130.

Ritchie CW, Bush AI, Mackinnon A, Macfarlane S, Mastwyk M, MacGregor L, Kiers L, Cherny R, Li Q-X, Tammer A, Carrington D, Mavros C, Volitakis I, Zilinas M, Ames D, Davis S, Beyreuther K, Tanzi RE, Masters CL (2003) Metal-protein attenuation with iodochlorhydroxyquin (clioquinol) targeting Aβ amyloid deposition and toxicity in Alzheimer's disease. Arch Neurol 60:1685-1691.

Roberson ED, Scearce-Levie K, Palop JJ, Yan F, Cheng IH, Wu T, Gerstein H, Yu G-Q, Mucke L (2007) Reducing endogenous tau ameliorates amyloid β-induced deficits in an Alzheimer's disease mouse model. Science 316:750-754.

Rockenstein E, Mallory M, Mante M, Sisk A, Masliah E (2001) Early formation of mature amyloid-β protein deposits in a mutant APP transgenic model depends on levels of Aβ1-42. J Neurosci Res 66:573-582.

Rockenstein E, Adame A, Mante M, Moessler H, Windisch M, Masliah E (2003) The neuroprotective effects of Cerebrolysin™ in a transgenic model of Alzheimer's disease are associated with improved behavioural performance. J Neural Transm 110:1313-1327.

Rockenstein E, Torrance M, Mante M, Adame A, Paulino A, Rose JB, Crews L, Moessler H, Masliah E (2006) Cerebrolysin decreases amyloid-β production by regulating amyloid protein precursor maturation in a transgenic model of Alzheimer's disease. J Neurosci Res 83:1252-1261.

Rockenstein E, Torrance M, Adame A, Mante M, Bar-on P, Rose JB, Crews L, Masliah E (2007) Neuroprotective effects of regulators of the glycogen synthase kinase-3β signalling pathway in a transgenic model of Alzheimer's disease are associated with reduced amyloid precursor protein phosphorylation. J Neurosci 27:1981-1991.

Rodi DJ, Janes RW, Sanganee HJ, Holton RA, Wallace BA, Makowski L (1999) Screening of a library of phage-displayed peptides identifies human Bcl-2 as a taxol-binding protein. J Mol Biol 285:197-203.

Roher AE, Chaney MO, Kuo Y-M, Webster SD, Stinel WB, Haverkamp LJ, Woods AS, Cotter RJ, Tuohy JM, Krafft GA, Bonnell BS, Emmerling MR (1996) Morphology and toxicity of Aβ-(1-42) dimer derived from neuritic and vascular amyloid deposits of Alzheimer's disease. J Biol Chem 271:20631-20635.

Rohn TT, Head E, W.H. N, Cotman CE, Cribbs DH (2001a) Activation of caspase-8 in the Alzheimer's disease brain. Neurobiol Dis 8:1006-1016.

Rohn TT, Head E, Su JH, Anderson AJ, Bahr BA, Cotman CW, Cribbs DH (2001b) Correlation between caspase activation and neurofibrillary tangle formation in Alzheimer's disease. Amer J Pathol 158:189-198.

Rohn TT, Rissman RA, Davis MC, Kim YE, Cotman CW, Head E (2002) Caspase-9 activation and caspase cleavage of tau in the Alzheimer's disease brain. Neurobiol Dis 11:341-354.

Rosario ER, Carroll JC, Oddo S, LaFerla FM, Pike CJ (2006) Androgens regulate the development of neuropathology in a triple transgenic mouse model of Alzheimer's disease. J Neurosci 26:13384-13389.

Rosenfeld J, Dorman ME, Griffin JW, Sternberger LA, Sternberger NH, Price DL (1987) Distribution of neurofilament antigens after axonal injury. J Neuropathol Exp Neurol 46:269-282.

Roth KA (2001) Caspases, apoptosis, and Alzheimer's disease: causation, correlation, and confusion. J Neuropathol Exp Neurol 60:829-839.

Roy M, Sapolsky RM (2003) The exacerbation of hippocampal excitotoxicity of glucocorticoids is not mediated by apoptosis. Neuro-endo 77:24-31.

Rozemuller AJM, Van Gool WA, Eikelenboom P (2005) The neuroinflammatory response in plaques and amyloid angiopathy in Alzheimer's disease: therapeutic implications. Curr Drug Targets 4:223-233.

Ruether E, Alvarez XA, Rainer M, Moessler H (2002) Sustained improvement of cognition and global function in patients with moderately severe Alzheimer's disease: a double-blind, placebo-controlled study with the neurotrophic agent Cerebrolysin. J Neural Transm 62:265-275.

Ruther E, Ritter R, Apecechea M, Freytag S, Gmeinbauer R, Windisch M (2000) Sustained improvements in patients with dementia of Alzheimer's type (DAT) 6 months after termination of Cerebrolysin therapy. J Neural Transm 107:815-829.

Ryder J, Su Y, Liu F, Li B, Zhou Y, Ni B (2003) Divergent roles of GSK3 and CDK5 in APP processing. Biochem Biophys Res Comm 312:922-929.

Sabo SL, Ikin AF, Buxbaum JD, Greengard P (2003) The amyloid precursor protein and its regulatory protein, FE65, in growth cones and synapses in vitro and in vivo. J Neurosci 23:5407-5415.

Sadowski M, Pankiewicz J, Scholtzova H, Ji Y, Quartermain D, Jensen CH, Duff K, Nixon RA, Gruen RJ, Wisniewski T (2004) Amyloid-beta deposition is associated with decreased hippocampal glucose metabolism and spatial memory impairment in APP/PS1 mice. J Neuropathol Exp Neurol 63:418-428.

Sahuquillo J, Poca MA (2002) Diffuse axonal injury after head trauma. A review. Adv Tech Stand Neurosurg 27:23-86.

Sampson VL, Morrison JH, Vickers JC (1997) The cellular basis of the relative resistance of parvalbumin and calretinin immunoreactive neocortical neurons to the pathology of Alzheimer's disease. Exp Neurol 145:295-302.

Sandbrink R, Beyreuther K (1996) Unraveling the molecular pathway of Alzheimer's disease: research about presenilins gathers momentum. Molecular Psychiatry 1:438-444.

SantaCruz K, Lewis J, Spires T, Paulson J, Kotilinek L, Ingelsson M, Guimaraes A, DeTure M, Ramsden M, McGowan E, Forster C, Yue M, Orne J, Janus C, Mariash A, Kuskowski M, Hyman B, Hutton M, Ashe KH (2005) Tau suppression in a neurodegenerative mouse model improves memory function. Science 309:476-481.

Sasaki A, Shoji M, Harigaya Y, Kawarabayashi T, Ikeda M, Naito M, Matsubara E, Abe K, Nakazato Y (2002) Amyloid cored plaques in Tg2576 transgenic mice are characterized by giant plaques, slightly activated microglia, and the lack of paired helical filament-typed, dystrophic neurites. Virchows Arch 441:358-367.

Satou T, Cummings BJ, Cotman CW (1995) Immunoreactivity for Bcl-2 protein within neurons in the Alzheimer's disease brain increases with disease severity. Brain Res 687:35-43.

Saunders HL, Dickson TC, Vickers JC (1998) Immonolocalisation of beta-amyloid precursor protein to plaque-associated synaptic alterations in the early and late stages of Alzheimer's Disease. Alzheimer's Reports 1:1-5.

Savonenko A, Xu GM, Melnikova T, Morton JL, Gonzales V, Wong MPF, Price DL, Tang F (2005) Episodic-like memory deficits in the APPswe/PS1dE9 mouse model of Alzheimer's disease: relationships to β-amyloid deposition and neurotransmitter abnormalities. Neurobiol Dis 18:602-617.

Sawa A, Oyama F, Cairns NJ, Amano N, Matsushhita M (1997) Aberrant expression of bcl-2 gene family in Down's syndrome brain. Mol Brain Res 48:53-59.

Schaefer EJ, Beiser AS, Lamon-Fava S, Robins SJ, Au R, Tucker KL, Kyle DJ, Wilson PW, Wolf PA (2006) Plasma phosphatidylcholine docosahexaenoic acid content and risk of dementia and Alzheimer's disease. Arch Neurol 63:1545-1550.

Schechter R, Yen S-HC, Terry RD (1981) Fibrous astrocytes in senile dementia of the Alzheimer type. J Neuropathol Exp Neurol 40:95-101.

Schenk D, Barbour R, Dunn W, Gordon G, Grajeda H, Guido T, Hu K, Huang J, Johnson-Wood K, Khan K, Kholodenko D, Lee M, Liao Z, Lieberburg I, Motter R, Mutter L, Soriano F, Shopp G, Vasquez N, Vandevert C, Walker S, Wogulis M, Yednock T, games D, Seubert P (1999) Immunization with amyloid-β attenuates Alzheimer-disease-like pathology in the PDAPP mouse. Nature 400: 173-177.

Schmitt AB, Breuer S, Voell M, Schwaiger F-W, Spitzer C, Pech K, Brook GA, Noth J, Kreutzberg GW, Nacimiento W (1999) GAP43 (B-50) and C-Jun are up-regulated in axotomized neurons of Clarke's nucleus after spinal cord injury in the adult rat. Neurobiol Dis 6:122-130.

Schultz JG, Salzer U, Mohajeri MH, Franke D, Heinrich J, Pavlovic J, Wollmer MA, Nitsch RM, Moelling K (2004) Antibodies form a DNA peptide vaccination decrease the brain amyloid burden in a mouse model of Alzheimer's disease. J Mol Med 82:706-714.

Schwab C, Hosokawa M, McGeer PL (2004) Transgenic mice overexpressing amyloid beta protein are an incomplete model of Alzheimer disease. Exp Neurol 188:52-64.

Schwerk C, Schulze-Osthoff K (2003) Non-apoptotic functions of caspases in cellular proliferation and differentiation. Biochem Pharmacol 66:1453-1458.

Selkoe DJ (1996) Amyloid beta-protein and the genetics of Alzheimer's disease. J Biol Chem 271:18295-18298.

Selznick LA, Timothy BS, Zheng S, Flavell RA, Rakic P, Roth KA (2000) Amyloid beta-induced neuronal death is Bax-dependent but caspase-independent. J Neuropathol Exp Neurol 59:271-279.

Sheng JG, Mrak RE, Sue W, Griffin T (1998) Progressive neuronal DNA damage associated with neurofibrillary tangle formation in Alzheimer's disease. J Neuropathol Exp Neurol 57:323-328.

Shepherd CE, Gregory GC, Vickers JC, Brooks WS, Kwok JBJ, Schofield PR, Kril JJ, Halliday GM (2004) Positional effects of presenilin-1 mutations on tau phosphorylation in cortical plaques. Neurobiol Dis 15:115-119.

Shepherd CE, Gregory GC, Vickers JC, Halliday GM (2005) Novel 'inflammatory plaque' pathology in presenilin-1 Alzheimer's disease. Neuropath App Neurobiol 31:503-511.

Shimohama S (2000) Apoptosis in Alzheimer's disease-an update. Apoptosis 5:9-16.

Shoghi-Jadid K, Small GW, Agdeppa ED, Kepe V, Ercoli LM, Siddarth P, Read S, Satyamurthy N, Petric A, Huang SC, Barrio JR (2002) Localization of neurofibrillary tangles and beta-amyloid plaques in the brains of living patients with Alzheimer disease. Am J Geriatr Psychiatry 10:24-35.

Shumaker SA, Legault C, Rapp SR, Thal L, Wallace RB, Ockene JK, Hendrix SL, Jones BN 3rd, Assaf AR, Jackson RD, Kotchen JM, Wassertheil-Smoller S, Wactawski-Wende J; WHIMS Investigators (2003) Estrogen plus progestin and the incidence of dementia and mild cognitive impairment in postmenopausal women: the Women's Health Initiative Memory Study: a randomized controlled trial. JAMA 289:2651-62.

Shumaker SA, Legault C, Kuller L, Rapp SR, Thal L, Lane DS, Fillit H, Stefanick ML, Hendrix SL, Lewis CE, Masaki K, Coker LH; Women's Health Initiative Memory Study (2004) Conjugated equine estrogens and incidence of probable dementia and mild cognitive impairment in postmenopausal women: Women's Health Initiative Memory Study. JAMA 291:2947-58.

Siemers ER, Skinner M, Dean RA, Gonzales C, Satterwhite J, Farlow M, Ness D, May PC (2005) Safety, tolerability, and changes in amyloid beta concentrations after administration of a gamma-secretase inhibitor in volunteers. Clin Neuropharmacol 28:126-132.

Siemers ER, Quinn JF, Kaye J, Farlow MR, Porsteinsson A, Tariot P, Zoulnouni P, Galvin JE, Holtzman DM, Knopman DS, Satterwhite J, Gonzales C, Dean RA, May PC (2006) Effects of a gamma-secretase inhibitor in a randomized study of patients with Alzheimer disease. Neurology 66:602-604.

Sigurdsson EM, Scholtzova H, Mehta PD, Frangione B, Wisniewski T (2001) Immunization with a nontoxic/nonfibrillar Amyloid-β homologous peptide reduces Alzheimer's disease associated pathology in transgenic mice. Amer J Pathol 159:439-447.

Simons M, Schwarzler F, Lutjohann D, Von Bergmann K, Beyreuther K, Dichgans J, Wormstall H, Hartmann T, Schulz JB (2002) Treatment with simvastatin in normocholesterolemic patients with Alzheimer's disease: a 26-week randomized, placebo-controlled, double-blind trial. Ann Neurol 52:346-350.

Singer O, Marr RA (2005) Targeting BACE1 siRNAs ameliorates Alzheimer disease neuropathology in a transgenic model. Nat Neurosci 8:1343-2349.

Sjogren M, Gustafsson K, Syversen S, Olsson A, Edman A, Davidsson P, Wallin A, Blennow K (2003) Treatment with simvastatin in patients with Alzheimer's disease lowers both alpha- and beta-cleaved amyloid precursor protein. Dement Geriatr Cogn Disord 16:25-30.

Smale G, Nichols N, Brady DR, Finch CE, Horton WE (1995) Evidence for apoptotic cell death in Alzheimer's disease. Exp Neurol 133:225-230.

Small GW, Kepe V, Ercoli LM, Siddarth P, Bookheimer SY, Miller KJ, Lavretsky H, Burggren AC, Cole GM, Vinters HV, Thompson PM, Huang S-C, Satyamurthy N, Phelps ME, Barrio JR (2006) PET of brain amyloid and tau in mild cognitive impairment. New Eng J Med 335:2652-2663.

Smith M, Perry G, Richey P, Sayre L, Anderson V, Beal M, Kowall N (1996) Oxidative damage in Alzheimer's. Nature 382:120-121.

Smith M, Harris P, Sayre L, Perry G (1997) Iron accumulation in Alzheimer's disease is a source of redox-generated free radicals. Proc Natl Acad Sci U S A 94:9866-9868.

Smith FM, Raghupathi R, MacKinnon M-A, McIntosh TK, Saatman KE, Meaney DF, Graham DI (2000) TUNEL-positive staining of surface contusions after fatal head injury in man. Acta Neuropathol 100:537-545.

Smith DH, Uryu K, Saatman KE, Trojanowski JQ, McIntosh TK (2003) Protein accumulation in traumatic brain injury. Neuromol Med 4:59-72.

Sola C, Garcia-Ladona FJ, Sarasa M, Mengod G, Probst A, Palacios G, Palacios JM (1993) βAPP gene expression is increased in the rat brain after motor neuron axotomy. Eur J Neurosci 5:795-808.

Sola S, Castro RE, Laires PA, Steer CJ, Rodrigues CMP (2003) Tauroursodeoxycholic acid prevents amyloid-β peptide-induced neuronal death via a phosphatidylinositol 3-kinase-dependent signaling pathway. Mol Med 9:226-234.

Sparks DL, Sabbagh MN, Conner DJ, Lopez J, Launer LJ, Browne P, Wasser D, Johnson-Traver S, Lochhead J, Ziolwolski C (2005) Atorvastatin for the treatment of mild to moderate Alzheimer's disease. Arch Neurol 62:753-757.

Spires TL, Meyer-Luehmann M, Stern EA, McLean PJ, Skoch J, Nguyen PT, Bacskai BJ, Hyman BT (2005) Dendritic spine abnormalities in amyloid precursor protein transgenic mice demonstrated by gene transfer and intravital multiphoton microscopy. J Neurosci 25:7278-7287.

Sponne I, Fifre A, Drouet B, Klein C, Koziel V, Pincon-Raymond M, Olivier J-L, Chambaz J, Pillot T (2003) Apoptotic neuronal cell death induced by non-fibrillar amyloid-β peptide proceeds through an early reactive oxygen species-dependent cytoskeletal perturbation. J Biol Chem 278:3437-3445.

Springer JE, Azbill RD, Knapp PE (1999) Activation of the caspase-3 apoptotic cascade in traumatic spinal cord injury. Nat Med 5:943-946.

St John PA (2007) Differential binding and activation of caspase-3 in cultured hippocampal neurons by assembly forms of Aβ1-42. J Neurosci Res 85:1205-1214.

Stadelmann C, Bruck W, Bancher C, Jellinger K, Lassmann H (1998) Alzheimer disease: DNA fragmentation indicates increased neuronal vulnerability, but not apoptosis. J Neuropathol Exp Neurol 57:456-464.

Stadelmann C, Deckwerth TL, Srinivasan A, Bancher C, Bruck W, Jellinger K, Lassmann H (1999) Activation of caspase-3 in single neurons and autophagic granules of granulovacular degeneration in Alzheimer's disease. Amer J Pathol 155:1459-1466.

Stefanis L (2005) Caspase-dependent and -independent neuronal death: two distinct pathways to neuronal injury. Neuroscientist 11:50-62.

Stein TD, Anders NJ, DeCarli C, Chan SL, Mattson MP, Johnson JA (2004) Neutralization of transthyretin reverses the neuroprotective effects of secreted amyloid precursor protein (APP) in APPSW mice resulting in tau phosphorylation and loss of hippocampal neurons: support for the amyloid hypothesis. J Neurosci 24:7707-7717.

Steiner H, Revesz T, Neumann M, Romig H, Grim MG, Pesold B, Kretzschmar HA, Hardy J, Holton JL, Baumeister R, Houlden H, Haass C (2001) A pathogenic presenilin-1 deletion causes abberrant Aβ42 production in the absence of congophilic amyloid plaques. J Biol Chem 276:7233-7239.

Stern EA, Bacskai BJ, Hickey GA, Attenello FJ, Lombardo JA, Hyman BT (2004) Cortical synaptic integration in vivo is disrupted by amyloid-β plaques. J Neurosci 24:4535-4540.

Stockley JH, O'Neill C (2007) The proteins BACE1 and BACE2 and beta-secretase activity in normal and Alzheimer's disease brain. Biochem Soc Trans 35:574-576.

Stone JR, Singleton RH, Povlishock JT (2001) Intra-axonal neurofilament compaction does not evoke local axonal swelling in all traumatically injured axons. Exp Neurol 172:320-331.

Strittmatter WJ, Roses AD (1995) Apolipoprotein E and Alzheimer disease. Proc Natl Acad Sci U S A 92:4725-4727.

Sturchler-Pierrat, Abramowski D, Duke M, Wierderhold K, Mistl C, Rothacher S, Ledermann B, Burki K, Frey P, Paganetti P, Waridel C, Calhoun M, Jucker M, Probst A, Staufenbeil M, Sommer B (1997) Two amyloid precursor protein transgenic mouse models with Alzheimer disease-like pathology. Proc Natl Acad Sci U S A 94:13287-13292.

Su JH, Anderson AJ, Cummings BJ, Botman CW (1994a) Immunohistochemical evidence for apoptosis in Alzheimer's disease. NeuroReport 5:2529-2533.

Su JH, Cummings BJ, Cotman CW (1994b) Early phosphorylation of tau in Alzheimer's disease occurs at Ser-202 and is preferentially located within neurites. NeuroReport 5:2358-2362.

Su JH, Cummings BJ, Cotman CW (1996a) Plaque biogenesis in brain aging and Alzheimer's disease. I. Progressive changes in states of paired helical filaments and neurofilaments. Brain Res 739:79-87.

Su JH, Satou T, Anderson AJ, Cotman CW (1996b) Up-regulation of Bcl-2 is associated with neuronal DNA damage in Alzheimer's disease. NeuroReport 7:437-440.

Su JH, Deng G, Cotman CW (1997) Bax protein expression is increased in Alzheimer's brain: correlations with DNA damage, Bcl-2 expression, and brain pathology. J Neuropathol Exp Neurol 56:86-93.

Su JH, Cummings BJ, Cotman CW (1998) Plaque biogenesis in brain aging and Alzheimer's disease. II. Progressive transformation and developmental sequence of dystrophic neurites. Acta Neuropathologica 96:463-471.

Su JH, Kesslak P, Head E, Cotman CW (2002) Caspase-cleaved amyloid precursor protein and activated caspase-3 are co-localized in the granules of granulovacular degeneration in Alzheimer's disease and Down's syndrome brain. Acta Neuropathol 104:1-6.

Su JH, Anderson AJ, Cribbs DH, Tu C, Tong L, Kesslack P, Cotman CW (2003) Fas and Fas ligand are associated with neuritic degeneration in the AD brain and participate in β-amyloid-induced neuronal death. Neurobiol Dis 12:182-193.

Suen K-C, Yu M-S, So K-F, Chang RC-C, Hugon J (2003) Upstream signaling pathways leading to the activation of double-stranded RNA-dependent serine/threonine protein kinase in b-amyloid peptide neurotoxicity. J Biol Chem 278:49819-49827.

Sugaya K, Reeves M, McKinney M (1997) Topographic association between DNA fragmentation and Alzheimer's disease neuropathology in the hippocampus. Neurochem Int 31:275-281.

Sullivan PG, Brown MR (2005) Mitochondrial aging and dysfunction in Alzheimer's disease. Prog Neuropsychopharmacol Biol Psychiatry 29:407-410.

Sung S, Yao Y, Uryu K, Yang H, Lee VM, Trojanowski JQ, Practico D (2004) Early vitamin E supplementation in young but not aged mice reduces Abeta levels and amyloid deposition in a transgenic model of Alzheimer's disease. FASEB J 18:323-325.

Swaab DF, Lucassen PJ, Salehi A, Scherder EJA, Van Someren EJW, Verwer RWH (1998) Reduced neuronal activity and reactivation in Alzheimer's disease. In: Progress in Brain Research (Van Leeuwen FW, Salehi A, Griger RJ, Holtmaat AJGD, Verhaagen J, eds), pp 343-377. Amsterdam: Elsevier Science Publishers.

Sykova E, Vorisek I, Antonova T, Mazel T, Meyer-Luehmann M, Jucker M, Hajek M, Ort M, Bures J (2005) Changes in extracellular space size and geometry in APP23 transgenic mice: a model of Alzheimer's disease. Proc Natl Acad Sci U S A 102:479-484.

Szpak GM, Lechowicz W, Lewandowska E, Bertrand E, Wierzba-Bobrowicz T, Gwiazda E, Schmidt-Sidor B, Dymecki J (2001) Neurones and microglia in central nervous system immune response to degenerative processes. Part 1: Alzheimer's

disease and Lewy body variant of Alzheimer's disease. Quantitative study. Folia Neuropathol 39:181-192.

Tabira T, Chui DH, Nakayama H, Kuroda S, Shibuya M (2002) Alzheimer's disease with spastic paresis and cotton wool type plaques. J Neurosci Res 70:367-372.

Takao M, Ghetti B, Hayakawa I, Ikeda E, Fukuuchi Y, Miravalle L, Piccardo P, Murrell JR, Glazier BS, Koto A (2002) A novel mutation (G217D) in the presenilin 1 gene (PSEN1) in a Japanese family: presenile dementia and parkinsonism are associated with cotton wool plaques in the cortex and striatum. Acta Neuropathol 104:155-170.

Takeuchi A, Irizarry MC, Duff K, Saido TC, Ashe KH, Hasegawa M, Mann DMA, Hyman BT, Iwatsubo T (2000) Age-related amyloid-β deposition in transgenic mice overexpressing both Alzheimer mutant presenilin 1 and amyloid-β precursor protein Swedish mutant is not associated with global neuronal loss. Amer J Pathol 157:331-339.

Tanzi RE (1999) Caspases land on APP: One small step for apoptosis, on giant leap for amyloidosis? Nat Neurosci 2:585-586.

Tatton WG, Chlamers-Redman R, Brown D, Tatton N (2003) Apoptosis in Parkinson's disease: signals for neuronal degradation. Ann Neurol 53:S61-S72.

Terry RD, Masliah E, Salmon SP, Butters N, DeTeresa R, Hill R, Hansen LA, Katzman R (1991) Physical basis of cognitive alterations in Alzheimer's disease: synapse loss is the major correlate of cognitive impairment. Ann Neurol 30:572-580.

Terry R (1996) The pathogenesis of Alzheimer's disease: an alternative to the amyloid hypothesis. J Neuropathol Exp Neurol 55:1023-1025.

Terwel D, Lasrado R, Snauwaert J, Vandeweert E, Van Haesendonck C, Boghgraef P, Van Leuven F (2005) Changed conformation of mutant tau-P301L underlies the moribund tauopathy, absent in progressive, nonlethal axonopathy of tau-4R/2N transgenic mice. J Biol Chem 280:2963-2973.

Teter B, Xu P-T, Gilbert JR, Roses AD, Galasko D, Cole GM (1999) Human apoplipoprotein E isoform-specific differences in neuronal sprouting in organotypic hippocampal culture. J Neurochem 73:2613-2616.

Thal DR, Glas A, Schneider W, Schober R (1997) Differential pattern of β-amyloid, amyloid precursor protein and apoplipoprotein E expression in cortical senile plaques. Acta Neuropathol 94:255-265.

Thal DRA, T. Waldmann, G. Holzer, M. Zedlick, D. Rub, U. Schober, R. (1998) Progression of neurofibrillary changes and PHF-tau in end-stage Alzheimer's disease is different from plaque and cortical microglial pathology. Neurobiol Aging 19:517-525.

Tomidokoro Y, Harigaya Y, Matsubara E, Ikeda M, Kawarabayashi T, Shirao T, Ishiguro K, Okamoto K, Younkin SG, Shoji M (2001) Brain Aβ amyloidosis in APPsw mice induces accumulation of presenilin-1 and tau. J Pathol 193:500-506.

Tompkins MM, Basgall EJ, Zamini E, Hill WD (1997) Apoptotic-like changes in Lewy-body-associated disorders and normal aging in substantia nigral neurons. Amer J Pathol 150:199-131.

Tortosa A, Lopez E, I. F (1998) Bcl-2 and Bax protein expression in Alzheimer's disease. Acta Neuropathol 95:407-412.

Trinchese F, Liu S, Battaglia F, Walter S, Mathews PM, Arancio O (2004) Progressive age-related development of Alzheimer-like pathology in APP/PS1 mice. Ann Neurol 55:801-814.

Trojanowski JQ, Lee VM (1994) Paired helical filament tau in Alzheimer's disease. The kinase connection. Amer J Pathol 144:449-453.

Troncoso JC, Sukhov RR, Kawas CH, Koliatsos VE (1996) In situ labelling of dying cortical neurons in normal aging and in Alzheimer's disease: correlations with senile plaques and disease progression. J Neuropathol Exp Neurol 55:1134-1142.

Tsai J, Grutzendler J, Duff K, Gan W-B (2004) Fibrillar amyloid deposition leads to local synaptic abnormalities and breakage of neuronal branches. Nat Neurosci 7:1181-1183.

Tseng H-C, Zhou Y, Shen Y, Tsai L-H (2002) A survey of cdk5 activator p35 and p25 levels in Alzheimer's disease brains. FEBS Letters 523:58-62.

Tuszynski MH, Thal L, Pay M, Salmon DP, Sang H, Bakay R, Patel P, Blesch A, Vahlsing HL, Ho G, Tong G, Potkin SG, Fallon J, Hansen L, Mufson EJ, Kordower

JH, Gall C, Conner J (2005) A phase 1 clinical trial of nerve growth factor gene therapy for Alzheimer's disease. Nat Med 11:551-555.

Tuszynski MH (2007) Nerve growth factor gene delivery: animal models to clinical trials. Dev Neurobiol 67:1204-1215.

Ujiie M, Dickenstein DL, Carlow DA, Jefferies WA (2003) Blood-brain barrier permeability precedes senile plaque formation in an Alzheimer's disease model. Microcirculation 10:463-470.

Van Dam D, D'Hooge R, Staufenbiel M, Binneken CV, Van Meir F, Deyn PPD (2003) Age-dependent cognitive decline in the APP23 model precedes amyloid deposition. Eur J Neurosci 17:388-396.

Van Leeuwen FW, Burbach JP, Hol EM (1998) Mutations in RNA: a first example of molecular misreading in Alzheimer's disease. Trends Neurosci 21:331-335.

Vasak M (2005) Advances in metallothionein structure and functions. J Trace Elem Med Biol 19:13-17.

Verdaguer E, Garcia-Jorda E, Canudas AM, Dominguez E, Jimenez A, Pubill D, Escubedo E, Pallas JCM, Camins A (2002) Kainic acid-induced apoptosis in cerebellar granule neurons: an attempt at cell cycle re-entry. NeuroReport 13:413-416.

Vickers JC, Delacourte A, Morrison JH (1992) Progressive transformation of the cytoskeleton associated with normal aging and Alzheimer's disease. Brain Res 594:273-278.

Vickers JC, Riederer R, Marugg R, Buée-scherrer V, Buée L, Delacourtes A (1994) Alterations in neurofilament protein immunoreactivity in human hippocampal neurons related to normal aging and Alzheimer's Disease. Neuroscience 621:1-13.

Vickers JC, Chin D, Edwards A-M, Sampson V, Harper C, Morrison J (1996) Dystrophic neurite formation associated with age-related β-amyloid deposition in the neocortices: clues to the genesis of neurofibrillary pathology. Exp Neurol 141:1-11.

Vickers JC (1997) A cellular mechanism for the neuronal changes underlying Alzheimer's disease. Neurosci 78:629-639.

Vickers JC, Dickson TC, Adlard PA, Saunders HL, King CE, McCormack G (2000) The cause of neuronal degeneration in Alzheimer's disease. Prog Neurobiol 60:139-165.

Vickers JC (2001) Genetically engineered models of human age-related neurodegenerative disease. In: Functional Neurobiology of Aging (Hof PR, Mobbs CV, eds), pp 387-406. San Diego: Academic Press.

Vickers JC, Tan A, Dickson TC (2003) Direct determination of the proportion of intra- and extra-cellular neocortical neurofibrillary tangles in Alzheimer's disease. Brain Res 971:135-137.

Vincent I, Jicha G, Rosado M, Dickson DW (1997) Aberrant expression of mitotic cdc2/cyclin B1 kinase in degenerating neurons of Alzheimer's disease brain. J Neurosci 17:3588-3598.

Von Gunten A, Kovari E, Bussière T, Rivara C-B, Gold G, Bouras C, Hof PR, Giannakopoulos P (2006) Cognitive impact of neuronal pathology in the entorhinal cortex and CA1 field in Alzheimer's disease. Neurobiol Aging 27:270-277.

Walsh DM, Hartley DM, Kusumoto Y, Fezoui Y, Condron MM, Lomakin A, Benedek GB, Selkoe DJ, Teplow DB (1999) Amyloid β-protein fibrillogenesis: structure and biological activity of protofibrillar intermediates. J Biol Chem 274:25945-25952.

Walsh DM, Townsend M, Podlisny MB, Shankar GM, Fadeeva FV, El Agnaf O, Hartley DM, Selkoe DJ (2005) Certain inhibitors of synthetic amyloid beta-peptide (Abeta) fibrillogenesis block oligomerization of natural Abeta and thereby rescue long-term potentiation. J Neurosci 25:2455-2462.

Wang SS-S, Becerra-Arteaga A, Good TA (2002) Development of a novel diffusion-based method to estimate the size of the aggregated Aβ species responsible for neurotoxicity. Biotechnol Bioeng 80:50-59.

Wang J, Tanila H, Poulivali J, Kadish I, Van Groen T (2003a) Gender differences in the amount and deposition of amyloid-β in APPswe and PS1 double transgenic mice. Neurobiol Dis 14:318-327.

Wang X, Zhu S, Drozda M, Zhang W, Stavrovskaya IG, Catteneo E, Ferrante RJ, Kristal BS, Friedlander RM (2003b) Minocycline inhibits caspase-independent and -dependent mitochondrial cell death pathways in models of Huntington's disease. Proc Natl Acad Sci U S A 100:10483-10487.

Wang R, Yan H, Tang X-C (2006) Progress in studies of huperzine A, a natural cholinesterase inhibitor from Chinese herbal medicine. Acta Pharm Sincia 27:1-26.

Weggen S, Eriksen JL, Das P, Sagi SA, Wang R, Pietrzik CU, Findlay KA, Smith TE, Murphy MP, Bulter T, Kang DE, Marquez-Sterling N, Golde TE, Koo EH (2001) A subset of NSAIDs lower amyloidogenic Aβ42 independently of cyclooxygenase activity. Nature 414:212-216.

Wei W, Norton D, Wang X, Kusiak J (2002) Aβ 17-42 in Alzheimer's disease activates JNK and caspase-8 leading to neuronal apoptosis. Brain 125:2036-2043.

West MJ, Coleman PD, Flood DG, Troncoso JC (1994) Differences in the pattern of hippocampal neuronal loss in normal ageing and Alzheimer's disease. Lancet 344:769-772.

Westerman MA, Cooper-Blacketer D, Mariash A, Kotilinek L, Kawarabayashi T, Younkin LH, Carlson GA, Younkin SG, Ashe KH (2002) The relationship between Aβ and memory in the Tg2576 mouse model of Alzheimer's disease. J Neurosci 22:1858-1867.

Whalen BM, Selkoe DJ, Hartley DM (2005) Small non-fibrillar assemblies of amyloid beta-protein bearing the Arctic mutation induce rapid neurite degeneration. Neurobiol Dis 20:254-266.

White F, Nicoll JAR, Roses AD, Horsburgh K (2001) Impaired neuronal plasticity in transgenic mice expressing human apolipoprotein E4 compared to E3 in a model of entorhinal cortex lesion. Neurobiol Dis 8:611-625.

Wierenga CE, Bondi MW (2007) Use of functional magnetic resonance imaging in the early identification of Alzheimer's disease. Neuropsychol Rev 17:127-143.

Wilcock DM, DiCarlo G, Henderson D, Jackson J, Clarke K, Ugen KE, Gordon MN, Morgan D (2003) Intracranially administered anti-Aβ antibodies reduce β-amyloid deposition by mechansims both independent of and associated with microglial activation. J Neurosci 23:3745-3751.

Wilcock DM, Rojiani A, Rosenthal A, Subbarao S, Freeman MJ, Gordon MN, Morgan D (2004) Passive immunotherapy against Aβ in aged APP-transgenic mice reverses cognitive deficits and depletes parenchymal amyloid deposits in spite of increased vascular amyloid and microhaemorrhage. J Neuroinflamm.

Wilcock GK, Black S, Haworth J, Laughlin M, Hendrix S, Binger M, Zavitz K, Swaab E, Hobden AA (2005) Placebo-controlled, double-blind trial of the selective Aβ-42

lowering agent, Flurizan (MPC-7869 (R)-flurbiprofen) in patients with mild to moderate Alzheimer's disease. Alzheimer's Association International Conference on Prevention of Dementia: U S A, Washington DC.

Wingrave JM, Schaecher KE, Sribnick EA, Wilford GG, S.K. R, Hazen-Martin DJ, Hogan EL, Banik NL (2003) Early induction of secondary injury factors causing activation of calpain and mitochondria-mediated neuronal apoptosis following spinal cord injury. J Neurosci Res 73:95-104.

Wirths O, Multhaup G, Czech C, Blanchard V, Moussaoui S, Tremp G, Pradier L, Beyreuther K, Bayer TA (2001) Intraneuronal Abeta accumulation precedes plaque formation in beta-amyloid precursor protein and presenilin-1 double-transgenic mice. Neurosci Lett 306:116-120.

Wu C-K, Thal L, Pizzo D, Hansen L, Masliah W, Geula C (2005) Apoptotic signals within the basal forebrain cholinergic neurons in Alzheimer's disease. Exp Neurol 195:484-496.

Xie Y, Yao Z, Chai H, Wong W-M, Wu W (2003) Potential roles of Alzheimer precursor protein A4 and β-amyloid in survival and function of aged spinal motor neurons after axonal injury. J Neurosci Res 73:557-564.

Xie T, Tong L, McCann UD, Yuan J, Becker KG, Mechan AO, Cheadle C, Donovan DM, Ricaurte GA (2004) Identification and characterization of metallothionein-1 and -2 gene expression in the context of (±)3,4-methylenedioxymethamphetamine-induced toxicity to brain dopaminergic neurons. J Neurosci 24:7043-7050.

Yamaguchi U, Ishiguro K, Uchida T, Takashima A, Lemere CA, Imahori K (1996) Preferential labeling of Alzheimer neurofibrillary tangles with antisera for tau protein kinase (TPK) I/glycogen synthase kinase-3β and cyclin-dependent kinase 5, a component of TPK II. Acta Neuropathol 92:232-241.

Yamamoto N, Yokoseki T, Shibata M, Yamaguchi H, Yanagisawa K (2005) Suppression of Aβ deposition in brain by peripheral administration of Fab fragments of anti-seed antibody. Biochem Biophys Res Comm 335:45-47.

Yang Y, Mufson EJ, Herrup K (2003) Neuronal cell death is preceded by cell cycle events at all stages of Alzheimer's disease. J Neurosci 23:2557-2563.

Yanker BA, Caceres A, Duffy LK (1990) Nerve growth factor potentiates the neurotoxicity of β-amyloid. Proc Natl Acad Sci U S A 87:9020-9023.

Yao M, Nguyen T, Vi V, Pike CJ (2005) β-amyloid-induced neuronal apoptosis involves c-Jun N-terminal kinase dependent downregulation of Bcl-w. J Neurosci 25:1149-1158.

Yuan A, Rao MV, Sasaki T, Chen Y, Kumar A, Veeranna, Liem RKH, Eyer J, Peterson AC, Julien J-P, Nixon RA (2006) α-internexin is structurally and functionally associated with the neurofilament triplet proteins in the mature CNS. J Neurosci 26:10006-10019.

Yue X, Lu M, Lancaster T, Cao P, Honda S, Staufenbiel M, Harada N, Zhong Z, Shen Y, Li R (2005) Brain estrogen deficiency accelerates Abeta plaque formation in an Alzheimer's disease animal model. Proc Natl Acad Sci U S A. 102:19198-203.

Zambenedetti P, Giordano R, Zatta P (1998) Metallothioneins are highly expressed in astrocytes and microcapillaries in Alzheimer's disease. J Chem Neuroanat 15:21-26.

Zhang J, Wu X, Qin C, Qi J, Ma S, Zhang H, Kong Q, Chen D, Ba D, He W (2003) A novel recombinant adeno-associated virus vaccine reduces behavioral impairment and β-amyloid plaques in a mouse model of Alzheimer's disease. Neurobiol Dis 14:365-378.

Zhang B, Higuchi M, Yoshiyama Y, Ishihara T, Forman MS, Martinez D, Joyce S, Trojanowski JQ, Lee VM-Y (2004a) Retarded axonal transport of R406W mutant tau in transgenic mice with a neurodegenerative tauopathy. J Neurosci 24:4657-4667.

Zhang HY, Yan H, Tang XC (2004b) Huperzine A enhances the level of secretory amyloid precursor protein and protein kinase C-alpha in intracerebroventricular beta-amyloid-(1-40) infused rats and human embryonic kidney 293 Swedish mutant cells. Neurosci Lett 360:21-24.

Zhang B, Maiti A, Shively S, Lakhani F, McDonald-Jones G, Bruce J, Lee EB, Xie SX, Joyce S, Li C, Toleikis PM, Lee VM-Y, Trojanowski JQ (2004c) Microtubule-binding drugs offset tau sequesteration by stabilizing microtubules and reversing fast axonal transport deficits in a tauopathy model. Proc Natl Acad Sci U S A 102:227-231.

Zhao M, Cribbs DH, Anderson AJ, Cummings BJ, Su JH, Wasserman AJ, Cotman CW (2003a) The induction of the TNF[alpha] death domain signaling pathway in Alzheimer's disease brain. Neurochem Res 28:307-318.

Zhao M, Su J, Head E, Cotman CW (2003b) Accumulation of caspase cleaved amyloid precursor protein represents an early neurodegenerative event in aging and in Alzheimer's disease. Neurobiol Dis 14:391-401.

Zhao H, Yenari MA, Cheng D, Sapolsky RM, Steinberg GK (2005) Biphasic cytochrome c release after transient global ischaemia and its inhibition by hypothermia. J Cereb Blood Flow Metab 25:1119-1129.

Zheng H, Koo EH (2006) The amyloid precursor protein: beyond amyloid. Mol Neurodegen 1:5.

Zhu S, Stavrovskaya IG, Drozda M, Kin BYS, Ona V, Li M, Sarang S, Liu AS, Hartley DM, Wu DC, Gullans S, Ferrante RJ, Przedhorski S, Kristal BS, Friedlander RM (2002) Minocycline inhibits cytochrome c release and delays progression of amyotrophic lateral sclerosis in mice. Nature 417:74-78.

Zipser BD, Johanson CE, Gonzalez L, Berzin TM, Tavares R, Huletter CM, Vitek MP, Hovanesian V, Stopa EG (2007) Microvascular injury and blood-brain barrier leakage in Alzheimer's disease. Neurobiol Aging 28:977-986.

9 APPENDIX – SOLUTIONS

0.01M PBS, pH 7.4

100mL 9% NaCl (90g of NaCl (Sigma) per 1L Milli-Q® water)

40mL Na_2HPO_4 (BDH Laboratory supplies, Poole, UK) (28.4g per 1L Milli-Q® water)

10mL $NaH_2PO_4.2H_2O$ (Sigma) (31.2g per 1L Milli-Q® water)

850mL Milli-Q® water

18.0% Sucrose Solution

180g Sucrose (Sigma) dissolved in 1L 0.01M PBS

30.0% Sucrose Solution

300g Sucrose (Sigma) dissolved in 1L 0.01M PBS

Tissue Storage Solution

0.01M PBS

0.1% Sodium azide (Sigma)

IMMUNOHISTOTOCHEMISTRY SOLUTIONS

0.25% Potassium permanganate

0.125g $KMnO_4$ (BDH) in 50mL 0.01M PBS

1.0% Pot-metabisulphite and oxalic acid

0.5g $K_2S_2O_5$ (BDH)

0.5g Oxalic acid (Analytic Univar Reagents, Victoria, Australia)

Dissolve in 50mL 0.01M PBS

0.0125% thioflavine s

0.00625g thioflavine s (Sigma) in 50mL 0.01M PBS

0.3% Triton/PBS

600μL Triton X (Sigma)

200mL 0.01M PBS

0.01M Citrate Buffer

2.94g Trisodium citrate (Sigma) in 800mL Milli-Q® water

Dissolve and adjust to pH 6 with 0.1M citric acid, then make up to 1L with Milli-Q® water.

0.1M Citric acid

10.5g Citric acid (Sigma) in 400mL Milli-Q® water

Dissolve and adjust to pH 6.0 with 2M NaOH, then make up to 1L with Milli-Q® water.

2M Sodium Hydroxide

40.0g NaOH (Sigma) dissolved in 1L Milli-Q® water

4% Paraformaldehyde (PFA)

40g PFA (Sigma)

40g Sucrose (Sigma)

100mL 9% NaCl

400mL Na_2HPO_4

500mL $NaH_2PO_4.2H_2O$

Heat while stirring until dissolved in a fume hood.

1.0% Hydrogen peroxide in methanol

1mL 30.0% H_2O_2 (Sigma) diluted in 30mL methanol

50X TAE

242g Tris Base (Sigma)

57.1mL glacial acetic acid

100mL 0.5M EDTA pH8.0 (BDH)

Make up to 1L with Milli-Q® water

TE Buffer, pH 8.0

1.6g Tris base

0.5g EDTA (Sigma)

Dissolve and adjust to pH 8.0 with 2M NaOH, then make up to 1L with Milli-Q® water.

5X Running Buffer, pH 8.3

9g Tris base

43.2g Glycine (Bio-Rad)

3g SDS

Combine and add 600mL of Milli-Q® water. Store at 4°C. Prior to use combine 100mL Running Buffer with 400mL Milli-Q® water.

2.5% Agarose Gel

1.25g Agarose (Invitrogen)

1mL 50x TAE

50mL Milli-Q® water

Microwave for two minutes. Add 4µl of Ethidium Bromide swirl, pour. Run gel in 1x TAE.