EDINBURGH
UNIVERSITY
LIBRARY

Shelf Mark Theses Section 2

RAMSAY, M.Sc. 1999

30150 021000093

RAMSAY, Mark F.
M.Sc. 1999

UNIVERSITY OF EDINBURGH THESIS

Author (surname, initials): RAMSAY, Mark F. **Degree:** M.Sc. **Year:** 1999

This thesis is an unpublished typescript and the copyright is held by the author. *All persons consulting this thesis, or having copies made, must sign the Copyright Declaration below.*

Copying regulations: **(1)** This thesis may be copied in whole or in part for the use of individuals and for libraries wishing to add this thesis to their stock. *Copying must be done by Edinburgh University Library.*

Copyright Declaration: I undertake fully to observe the author's copyright in this thesis, not to publish the whole or any part of it without the author's written permission, and not to allow any other person to use any copy made for me.

Date	Name and Address (BLOCK CAPITALS)	Signature	Pages copied (if any)

A Longitudinal Study of Spatial Learning in the PDAPP mouse.

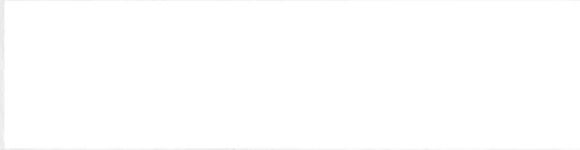
Mark F Ramsay

Submitted to The University of Edinburgh for the degree of MSc (Res)

December, 1998

Declaration

In accordance with The University of Edinburgh postgraduate regulation 3.8.7, I declare that this thesis was composed by myself, and the work presented herein is my own.



Mark F Ramsay

Dedication.

To my parents.

Acknowledgements

I would like to thank my supervisor, Professor Richard Morris, for providing me the opportunity to conduct the research in his laboratory and for constant help and advice throughout; Dora Games of Athena Neurosciences for supplying the mice and antibodies; Alan Justice of Athena Neurosciences for advice about experimental design and discussion of results; Jane Knox (University of Edinburgh) and Karen Khan (Athena Neurosciences) for technical assistance with the immunohistology; and Andrew Bernard and Jean Hunter for care of the animals. Finally, to other members of the lab -- Steve Martin, Bob Steele, Livia de Hoz and David Foster for their constant encouragement and support.

Contents.

Declaration 2

Dedication 3

Acknowledgements 4

Abstract 7

1 Background 8

2 Main Features of Alzheimer's Disease 10

2.1 Clinical Aspects 10

2.2 Pathological Markers 11

3 Main Areas or Research 14

3.1 Introduction 14

3.2 The Presenilins 14

3.3 Apolipoprotein E 18

3.4 Amyloid Hypothesis 19

3.5 Cellular Processing of APP 22

4 Animal Models 28

4.1 Introduction 28

4.2 Nonhuman Primate Models 28

4.3 Rodent Models 29

4.4 Lesion Models 29

4.5 Infusion Models 30

4.6 Current Models 30

4.7 Transgenic Models 31

4.71 The PDAPP mouse 31

4.72 The Tg2576 Mouse 33

4.73 Carboxy-terminus APP Transgenic Mice 36

4.74 The SciosNova Mouse 39

4.75 APP14, APP22 and APP23 Mice 40

4.76 Summary 42

5 Behavioural Experiment **44**
5.1 Introduction 44
5.2 General Methods 45
5.20 Subjects 45
5.21 The Watermaze 46
5.22 Cue Training 48
5.23 Delayed Matching to Place 48
5.24 Experimental Design 49
5.25 Immunohistology 52
6 Results **54**
6.1 Behavioural Data 54
6.2 Statistical Analyses 54
6.3 Cue Training 55
6.4 Delayed Matching to Place Training 60
6.5 Histology 69
7 Conclusions **72**
8 Discussion **73**
9 References **76**

Abstract.
Dementia is a psychiatric disorder of old age. Alzheimer's Disease (AD) is the most common form, accounting for 50-70% of all cases. In Western Europe, approximately 5% of the population over 60 years of age are affected, and this rises to 20% of the population aged 80 years or over. With the increase in the average length of life due to medical advances, the size of the aged population is rising and globally may number 1 billion by 2025. Thus, the number of AD cases is set to increase dramatically.

One approach to understanding a disease is to model it. This allows specific predictions to be tested and novel information about the basic mechanisms, cause, onset and progression of the disease to be elucidated. Recently, several transgenic mouse models of AD have been developed. One such model, the PDAPP mouse, shows AD-like pathology. The transgene is human amyloid precursor protein (APP), a protein implicated in AD, with a mutation found in families with inherited AD.

Spatial memory was investigated in this model as it is hippocampal dependent, and the hippocampal formation is one of the earliest areas to be affected by AD. A longitudinal study in the watermaze was undertaken to follow the performance of the animals as they aged. A delayed matching to place protocol was used due to repeated testing of the same animals at different ages.

PDAPP animals were impaired relative to littermate controls at the earliest age tested, and this deficit persisted as the animals aged. Consequently, no age-dependent deficit was observed. Mere overexpression of the transgene may be enough to cause a deficit in spatial learning, although the task may yet be improved in sensitivity to reveal an age-related change in performance.

1. Background.

Dementia is a psychiatric disorder of old age. Alzheimer's Disease (AD) is the most common form with estimates varying between 50% and 70% of all cases (Henderson 1986; Goldman and Cote 1991). Its incidence rises sharply with age: in Western Europe, for example, approximately 5% of the population have the disease at age 65 years, rising to 15% - 20% at age 80 or above (Lannfelt, Folkesson et al. 1993; Huppert and Tym 1986). Dementia has been defined as "the global deterioration of the individual's intellectual, emotional, and cognitive facilities in a state of unimpaired consciousness" (Roth 1981). Within this context, AD is classified as a disorder which has an insidious onset, is progressive and occurs in the absence of other systemic or brain diseases that would account for the progressive cognitive deficit and personality change (McKhann, Drachman et al. 1984).

The first use of the term senile dementia occurred in 1835. James C. Prichard used this term to describe a syndrome (which he also called incoherence) characterised by "forgetfulness of recent impressions, while the memory retains a comparatively firm hold of ideas laid up in the recesses from times long past." cited in (Henderson 1986). In 1907, Alois Alzheimer described the behaviour of a 51 year old patient which could not be categorised with respect to any known diseases at that time (Alzheimer 1907), see also (Wilkins and Brody 1969) for translation. He noted a rapidly increasing loss of memory, disorientation and auditory hallucinations. Her condition deteriorated until she died, 4.5 years later, prior to which she was bedridden and "completely stuporous". He was the first to describe the pathological hallmarks of the disease which now bears his name, namely plaques ("Scattered throughout the entire cortex, especially in the upper layers, one found miliary foci that were caused by the deposition of a peculiar substance in the cerebral cortex.") and tangles ("In the interior of a cell...one or several fibrils stood out due to their extra-ordinary

thickness...Then they merged into dense bundles and gradually reached the surface of the cell").

AD already affects a large number of people (one estimate is that 17-20 million people are affected world-wide) and is set to become more common as the population ages. Medical advances have eliminated premature death, particularly in developed countries, with the result that the average length of life has risen (in the US it has increased from 47 years at the beginning of the 20th century to 73 years now (Fries 1980)). One estimate puts the global population of people aged 60 or over in 1954 at 214 million with this rising to 1 billion by 2025 (Henderson 1986), although many calculate this figure to be much higher. Thus, the number of cases of AD is set to increase dramatically. It is the nature of the disease which makes these figures so striking. While in the early stages, there is some short-term memory loss and slight disorientation, in the late stages the subject is bed-ridden and needs 24hr care. Additionally, improved medical practices paradoxically exacerbate the problems because those with the disease are living longer. The socio-economic impact of AD is already large and will continue to grow at a rapid rate.

The disease can be divided into two main areas: sporadic AD and familial AD (FAD). Sporadic AD accounts for the vast majority of cases and has an onset of 65 years or greater. The cause remains unknown. Familial AD, named because it runs in family groups (i.e. it is hereditary), is caused by different mutations in different genes and has a much earlier age of onset, ranging from about 40 years to about 60 years (Hardy 1994).

2. Main Features of AD.

2.1 Clinical Aspects.

AD has a very diverse number of clinical manifestations reflecting the functions of different regions of the brain that are affected. At the present time, the only definitive diagnosis of the disease involves a post-mortem examination of the brain to discover if the characteristic pathological markers, neuritic (or senile) plaques and neurofibrillary, tangles are present. However, to facilitate a practical clinical classification of the disease, a 'working' nomenclature has been proposed (McKhann, Drachman et al. 1984). It consists of 3 levels: possible, probable and definite. A diagnosis of possible AD is given if the presentation or the time course is suggestive but does not follow the 'classical' path of AD; or in cases where the individual has another disease but AD is thought to be the cause of dementia. Probable AD is diagnosed if the disease had the typical insidious onset of AD and had been progressive, provided other causes have been excluded. A diagnosis of definite AD requires histological examination.

This classification is based on the cognitive, behavioural and neurological changes in AD. In the early stages of the disease, loss of recent memory is usually seen first. The individual may have an appreciation that they have a memory problem and may invent excuses to cover this fact. Concentration becomes impaired and fatigue becomes more frequent. The individual may become restless and anxious and in some cases even depression is seen although this is usually brief and does not occur without the other features of dementia. Personality still remains intact at this stage, although exaggerations of certain personality traits may appear. Intellectual impairment is usually not obvious.

In the intermediate stage of AD, all aspects of memory fail progressively. Parietal lobe deficits such as agnosia and dyspraxia appear. In 5-10% of cases, epileptic fits occur. Emotions become less marked and apathy becomes the dominant mood. The capacity for judgement, abstract thought and calculation have disappeared by this stage. Towards the end of this stage or later, a psychotic syndrome with auditory and/or visual hallucinations and delusion may happen.

In the late phase, all intellectual functions are grossly impaired. While the individual can still walk, the gait is unsteady and slow-moving. Marked emotional disinhibition occurs and the person's previous personality disappears. Patients cannot recognise close relatives or themselves. Eventually they become bedridden and become increasingly spastic and myoclonic. Double incontinence follows and a progressive wasting is seen despite an intact appetite. Eventually, an almost entirely vegetative state is seen, and some individuals may survive for several years in this condition.

2.2 Pathological Markers.

The neuritic plaque is a complex multicellular lesion (Selkoe 1994). The proteinaceous component is called amyloid which is deposited extracellularly. Amyloid is a 39-42 amino acid protein named amyloid-β peptide (Aβ) derived from a larger membrane-bound protein, amyloid precursor protein (APP). Aβ self-aggregates in a β-pleated sheet to form filaments approximately 8nm in length. Also involved in the plaque are dystrophic (dilated) and degenerating neurites (both axonal terminals and dendrites) and activated astrocytes and microglia. The arrangement of these components determines the morphology of the plaque.

'Classical' or neuritic plaques consist of an Aβ core (usually spherical in nature) surrounded by 5 or 6 microglial cells (Wisniewski and Wegiel 1994). Astrocytotic processes are found on the periphery of this structure. Where the amyloid filaments infiltrate the neuropil, the dystrophic and degenerating neurites are seen. These contain degenerating mitochondria, electron-dense bodies, neurofilaments and paired helical filaments (PHFs, see below).

Neuritic plaques account for only a minority of Aβ deposits in the brain. The majority are termed diffuse plaques. These deposits are roughly spherical in shape and are less dense than neuritic plaques. They rarely, if ever, contain dystrophic neurites and are associated with little or no activation of astrocytes and microglia. There is some evidence to suggest that these may develop into neuritic plaques although it is far from conclusive. In Down's Syndrome (trisomy 21, because individuals posses an extra copy of chromosome 21) diffuse plaques appear in the cortex after 20 years of age. These plaques are not associated with surrounding dystrophy, gliosis or tangle formation. Virtually all Down's patients over 50 years old have neuritic plaques, indicating that these diffuse deposits may change into denser neuritic plaques.

As mentioned above, reactive astrocytes and activated microglia are also associated with neuritic plaques. These cells produce compounds which mediate the local inflammatory response found round neuritic plaques and contribute to neurotoxicity. Some of the proteins that have been found associated with Aβ in plaques are α_1-antichymotrypsin, several components of the complement cascade (mediating an immune response), heparan sulphate proteoglycans and cytokines (Abraham, Selkoe et al. 1988; Snow, Mar et al. 1988; Rogers, Cooper et al. 1992). Pathology follows a certain pattern of expression in the brain. Layer II of the entorhinal cortex is the first area to be affected, followed by the hippocampus (especially the

pyramidal cells). The pathology spreads to the neocortex and in late stages of the disease, plaques can be found in the thalamus and striatum (Braak, Braak et al. 1996).

Neurofibrillary tangles (NFT) are the other neuropathological feature of AD described by Alzheimer (Alzheimer 1907; Wilkins and Brody 1969) and, are still recognised as a marker of AD (with plaques, as tangles are not unique to the disease). Tangles are accumulations of abnormal fibres seen in cell bodies and in some of the altered neurites of neuritic plaques. Electron microscopy has revealed that the main structures constituting the NFTs are pairs of twisted filaments arranged in a helix, 25nm across at its widest point and constricting every 80nm. These are called paired helical filaments (PHF) (Terry 1963). Also seen are 15nm long straight filaments. The component of PHFs was identified as the microtubule associated protein, tau (τ) after antibodies were raised to isolated PHFs (Nukina and Ihara 1986). Antibodies to PHFs or tau also detect a diffuse pattern of dystrophic cortical neurites that are not associated with plaques. These 'neuropil threads' appear to correlate with the presence of NFTs in the cortex (Probst, Anderson et al. 1989). Certain tau epitopes are abnormally phosphorylated, as revealed by the fact that some tau antibodies react with fibrillary lesions more strongly after *in vitro* dephosphorylation with alkaline phosphatase (Grunde-Iqbal, Iqbal et al. 1986).

PHFs are derived from the carboxyl third of tau, a region that includes the microtubule-binding domain (Wischik, Novak et al. 1988). They are often associated with ubiquitin but no proteins in the tangle that are modified by ubiquitination have yet identified and it is not clear if this type of proteolytic degradation is occurring. Cells with tangles eventually die, although it is not clear whether the accumulation of abnormally phosphorylated tau affects the function of normal tau or if the hyperphosphorylation is due to cytoskeletal reorganisation in dying neurons by another mechanism.

3. Main Areas of Research.

3.1 Introduction.

The discovery of certain mutated genes in familial populations has seen the field progress rapidly in the last 15 years. The fact that AD inevitably occurs in Down's syndrome (trisomy 21) and genetic linkage studies have revealed a genetic component to the disease. Once it was realised that AD was not genetically heterogeneous, mutations were found in several genes. These are the amyloid precursor protein (APP) gene on chromosome 21, presenilin 1 (PS 1) on chromosome 14, presenilin 2 (PS 2) on chromosome 1. In addition, a genetic 'risk factor' for late onset AD (occurring after 60 years of age) has been identified: the apolipoprotein E (ApoE) gene on chromosome 19. A brief description of the involvement of the presenilins and ApoE will be given before a more detailed description of the involvement of APP in the disease.

3.2 The Presenilins.

Presenilin 1 was identified using a positional cloning technique on a candidate region of chromosome 14 (Sherrington, Rogaev et al. 1995). Presenilin 2 was isolated because of its close homology to PS1 (67%) (Levy-Lahad, Wijsman et al. 1995). Both proteins are widely expressed in the nervous system and other peripheral tissues (Levy-Lahad, Wijsman et al. 1995; Sherrington, Rogaev et al. 1995). In rodent, primate and human brain, PSs are expressed at high levels in neurons, particularly in the pyramidal neurons of the hippocampus (Page, Hollister et al. 1996), and PS1 and PS2 are often found co-localised (Cook, Sung et al. 1996; Kovacks, Fausett et al. 1996; Lee, Slunt et al. 1996; Suzuki, Nishiyama et al. 1996). PS1 immunoreactivity is concentrated in cell bodies and dendrites, with lower levels present in axons (Elder, Tezapsidis et al. 1996; Lah, Heilman et al. 1997). The presenilins are putative transmembrane (TM) proteins with some TM domains bearing some homology to Ca^{2+} channels. These putative TM loops in PS1 and PS2 show very high sequence homology. The

predicted structure for PS1 has between 6 and 8 transmembrane loops, with both the N and C termini in the cytoplasm (Levy-Lahad, Wasco et al. 1995; Sherrington, Rogaev et al. 1995; Li and Greenwald 1996; Lehmann, Chiesa et al. 1997).

The functions of the presenilins have not yet been established but speculation as to their role(s) has come from information about their structure, cellular expression and subcellular localisation. Recently, it was discovered that the presenilins are homologues of two *Caenorhabditis elegans* genes: *sel*-12 (50% identity) and *spe*-4 (25% identity) (L'Hernault and Arduengo 1992; Levitan and Greenwald 1995). Mutations in *spe*-4 disrupt spermatogenesis through disruption of protein trafficking in the Golgi (L'Hernault and Arduengo 1992); those in *sel*-12 produce an egg-laying deficit, probably through disruption of the Notch signalling pathway. Patterns of expression of PS1 and Notch in the developing rodent nervous system are very similar, being high during neurogensis and decreasing as the embryo develops (Berezovska, Xia et al. 1997). PS 1 knockout mice show developmental abnormalities similar to those seen in mice in which other components of the Notch system have been knocked out (Wong, Zheng et al. 1997). Stronger evidence for a functional relationship between *sel*-12 and PS1 is provided by an elegant experiment showing that PS1 can rescue *sel*-12 mutant C. *elegans* (Levitan, Doyle et al 1996).

There have been 41 mutations identified in PS1 while only 2 in PS2 (see Hardy (1997) for a complete list). All the PS1 mutations but one are mis-sense mutations; the exception is an in-frame deletion in exon 9. These mutations are clustered in and near the putative transmembrane domains in both proteins. All the mis-sense mutations occur in amino acids that are conserved between PS1 and PS2. The mutations in PS1 appear to be 100% penetrant and are therefore classified as autosomal dominant 'causative' gene defects. They account for the largest population of FAD instances, being responsible for about 80% of cases. The mean onset of AD in families

with PS1 mutations is approximately 45 years old with a range of 32 to 56 years. This contrasts with the PS2 mutations, where the mean onset is 52 years with a range of 40-85 years. PS1 is proteolytically processed in a way that suggests there is a physiological role for such processing. For reviews, see Tanzi, Kovacks et al. 1996; Hardy 1997; Mattson and Guo 1997.

Currently, there are data supporting 3 different hypotheses of the mechanism by which mutations in the presenilins lead to plaque formation and synapse and neuronal death seen in AD: firstly, that PS mutations cause an alteration in APP processing which produces increased levels of Aβ, especially Aβ 1-42; secondly, that PS mutations promote apoptotic cell death pathways; and finally that the mutations cause aberrant ER calcium regulation which promotes excitotoxic and apoptotic cascades. These hypotheses are not mutually exclusive, and PS mutations may affect all of these mechanisms.

Mutations in APP are linked to cases of autosomal, dominant inherited AD (see below) and these mutations have been shown to produce the long form of Aβ (Aβ1-42) when expressed in cell culture or in transgenic models. This form of Aβ has been shown to seed amyloid aggregation (Younkin 1995). Additionally, Aβ1-42 appears to be the most common form of Aβ deposited in the brain in AD. Consequently, the effect of PS mutations on APP processing was tested. Plasma from subjects with PS mutations has been shown to have significantly elevated levels of Aβ1-42 and fibroblasts from these subjects release more of the long form of Aβ than from individuals without the mutation (Scheuner, Eckman et al. 1996). Furthermore, cultured cells and transgenic mice overexpressing mutant PS1 show increased levels of Aβ1-42 (Borchelt, Thinakaran et al. 1996; Duff, Eckman et al. 1996). It is possible that presenilins may affect APP processing either directly or indirectly.

Apoptosis and necrosis are two mechanisms of cell death. Generally, cells undergo apoptosis if they are exposed to subtle and prolonged exposure to adverse conditions and undergo necrosis if they are exposed to severe and sudden insults. Apoptosis appears to be amore controlled cell death with the requirement for particular proteins and the packaging of cell remnants. It has been reported that overexpression of mutant PS2 has an enhanced apoptotic effect to that of wild-type PS2 (Wolozin, Iwasaki et al. 1996). Possible involvement of PS1 has also been shown by the finding that expression of the mutant protein in cultured PC-12 cells sensitises them to apoptosis induced by Aβ (Guo, Furukawa et al. 1996).

It is also possible that PS mutations may enhance cell death by increasing levels of oxidative stress that the cell undergoes (see below for a more detailed description of oxidative stress in AD). Expression of mutant PS-1 in PC12 cells resulted in mitochondrial dysfunction and peroxide accumulation, which are indicators of peroxide accumulation (Guo, Sopher et al. 1997). This study also showed that antioxidants, such as vitamin E can protect the cell against the apoptotic actions of PS1.

Calcium homeostasis may also be affected by PS mutations. PC 12 cells harbouring mutant PS1 show increased calcium release from the endoplasmic reticulum (ER) when stimulated with carbachol or with bradykinin (Guo, Furukawa et al. 1996).

3.3 Apolipoprotein E.

Apolipoprotein E transports lipids, especially cholesterol in the central nervous system and in the periphery (Mahley 1998). The gene has three common alleles, designated ε2, ε3 and ε4. ε3 is the most common allele, accounting for 73% of all chromosomes; ε4 accounts for 15% and ε2 for 7%. In 1993, it was discovered that one of these alleles, ε4, was associated with late-onset (over 60 years of age) familial AD (Corder, Saunders et al. 1993; Strittmatter, Saunders et al. 1993). ε4 was found to be more common than normal in a group of unrelated subjects with familial AD, suggesting that it was contributing to the disease (Strittmatter, Saunders et al. 1993). This work was extended and confirmed for subjects with sporadic AD as well (Saunders, Strittmatter et al. 1993). There is a dose effect of the inheritance of the ε4 allele on the distribution of age of onset in familial AD. With each ε4 allele inherited, the risk of developing AD increases and the distribution of the age of onset decreases (Corder, Saunders et al. 1993). Inheriting the ε2 allele decreases the risk and increases the mean age of onset (Corder, Saunders et al. 1994). Thus ApoE4 has a major influence on AD. It must be stressed, however, that it is a risk factor, and so, unlike autosomal dominant or recessive traits, the mutation will not necessarily cause the disease.

ApoE has been found localised in plaques and in NFT (Namba, Tomonaga et al. 1991; Strittmatter, Saunders et al. 1993), leading to speculation that ApoE may mediate the β-pleated sheet formation that Aβ takes in plaques (Wisniewski and Frangione 1992). ApoE has been shown to bind to Aβ with high affinity *in vitro* (Wisniewski and Frangione 1992; Strittmatter, Saunders et al. 1993) and that lipid free E3 and E4 proteins form a stable complex with Aβ that cannot be degraded with sodium dodecyl sulphate, with E4 being more effective in forming the complex (Strittmatter, Weisgraber et al. 1993). Additionally, over several days, full-length, lipid-

free ApoE and Aβ incubations form insoluble, high-molecular weight complexes that adopt a fibril formation. E4 protein incubations formed a denser and more extensive collection of fibrils than E3. Furthermore, the fibrils appeared earlier with E4 (Sanan, Weisgraber et al. 1994).

In subjects with late-onset sporadic AD, individuals that are homozygous for the ε4 allele have increased vascular deposits and an increased number and density of neuritic plaques (Schmechel, Saunders et al. 1993). Thus, ApoE can bind to Aβ and influence its aggregation, with the E4 protein being more effective. This partially explains the increased risk of developing the disease with the ε4 allele.

3.4 The Amyloid Hypothesis

One of the main theories in explaining AD is the amyloid hypothesis. This draws from many lines of evidence, all which suggest that APP is involved in the disease. It states that deposition of Aβ is the central event in the disease and the other neuropathology is a consequence of this deposition. Mutations in APP and the presenilins lead to its mis-metabolism, producing the longer and more amyloidogenic form Aβ1-42 which aggregates as diffuse plaques. Aβ1-40 then aggregates onto these plaques. The plaques themselves evoke an inflammatory response, activating microglia and astrocytes, and releasing cytokines. Eventually, neuronal homeostasis breaks down, and oxidative injury becomes apparent. Kinase and phosphatase activity is altered, leading to hyperphosphorylated τ and the formation of PHF and tangles. Eventually widespread neuronal dysfunction and death occurs in the hippocampus and later in the cortex, leading to neurotransmitter deficits. The subject becomes demented. For reviews see (Selkoe 1994; Yanker 1996). Aβ itself has been shown to be toxic to cells (reviewed in Mattson 1997a), and to activate enzymes that produce reactive oxygen species (ROS) which are also toxic (see Mattson 1997b).

However, there are other arguments. Aβ deposition may not be the causative factor, but only the result of another mechanism. Some postulate that the accumulation of ROS as the brain ages is the real culprit and that this leads to Aβ deposition (reviewed in Benzi and Moretti 1995). As NFTs correlate more closely to the severity of the disease, others propose that AD due to alteration in the structure of the cell (Terry 1996). It may be that Aβ deposition is secondary to signal transduction disturbances (Fowler, Garlind et al. 1996; Fowler, Cowburn et al. 1997), which perturb synaptic plasticity, thereby altering processing.

It is generally accepted that all these events occur in the disease process, but their order and importance is disputed; does amyloid deposition lead to the other effects or do they cause the deposition? There is much evidence for the amyloid hypothesis, but this may be as a result of the APP mutations being the first discovered and so most work has concentrated on Aβ. The first animal models used mutant human APP and, even in the later presenilin models, altered APP processing is observed. As more is discovered about the disease, the situation may become more complicated before it gets clearer.

Aβ, the main component of amyloid plaques is derived from a larger protein, amyloid precursor protein (APP). APP was discovered after Glenner and Wong (1984) isolated and purified the subunit protein of the meningovascular amyloid filaments in AD and determined its amino-terminal sequence. They named the protein, which was approximately 4 kDa in size, amyloid β-peptide (Aβ). Following this, another study which partially purified neuritic plaques found in the cerebral cortex of AD found that the subunit of the amyloid plaque cores was the same peptide (Masters, Simmons et al. 1985). Using the partial sequence of Aβ, laboratories cloned cDNAs which encoded part or all of the precursor. The sequence obtained

from full length cDNA suggested the precursor was 695 amino acids in length, contained a single hydrophobic sequence near its C-terminus and had the properties of a membrane-spanning protein and contained a 17 amino acid long signal at the N-terminus for transport into the endoplasmic reticulum (Kang, Lemaire et al. 1987). The Aβ sequence began 28 residues amino-terminal to the single transmembrane domain and extended 11-15 residues into the domain.

Northern analysis and *in situ* hybridisation showed that APP was widely expressed in all neural and peripheral mammalian tissues, with the highest level of expression in the brain and kidney. In the brain, neurons showed very high levels of APP_{695}. In humans, the APP gene was found to be localised on chromosome 21 which provided evidence for its involvement in the formation of amyloid deposits in Down's syndrome (trisomy 21) as Down's subjects have an extra copy of this chromosome.

APP undergoes alternative splicing to produce three isoforms. The most common and widely expressed of these numbers 751 residues in length and contains a 56 amino acid insert in the extra-cellular portion of the protein. This insert has approximately 50% homology to the Kunitz family of serine proteases inhibitors (referred to as KPIs) (Tanzi, McClatchey et al. 1988). A longer, 770 residue protein also contains this exon plus another one adjacent to it of unknown function. APP_{695} does not contain either of these sequences. Other transcripts have been identified which comprise of a soluble, secreted form which contains the KPI domain but lacks the Aβ, the remaining transmembrane and cytoplasmic regions (de Sauvage and Octave 1989) and a form present in macrophage/microglial-type cells that lack the exon preceding the 2 exons which code for the Aβ region.

3.5 Cellular Processing of APP.

As mentioned above, there are different transcripts of APP. After synthesis on the rough endoplasmic reticulum, the protein undergoes a number of post-translation modification. In the Golgi, APP undergoes N-linked and then O-linked glycosylation (Weidemann, Konig et al. 1989). The ecto-and cytoplasmic domains are (Hung and Selkoe 1994; Suzuki, Ando et al. 1997; Walter, Capell et al. 1997). The function these modifications confers on the protein are unknown.

APP is cleaved by three enzymes, designated the α, β and γ secretases. The α secretase cleaves between amino acids 612 and 613 of APP (isoform 695 numbering) which corresponds to amino acids 16 and 17 of the Aβ sequence, thereby preventing formation of Aβ. The fragment released is named soluble APP (APP_s) as it does not form amyloid fibrils. Processing in this way is referred to as the secretory pathway. The amyloidogenic pathway involves the β and γ secretases. The former enzyme cleaves between amino acids 596 and 597 of APP, which corresponds the amino terminus of the Aβ sequence; γ secretase between amino acids 639 and 642 which corresponds to the carboxy terminus of the Aβ sequence. These action of these two enzymes results in liberation of Aβ varying in length from 39 to 42 amino acids. Several lines of evidence suggest that Aβ(1-42) aggregates more readily than the others and that this peptide is more neurotoxic (Jarrett, Berger et al. 1993; Suzuki, Cheung et al. 1994; Scheuner, Eckman et al. 1996).

Initially, it was thought that the secretory pathway was the normal physiological process and the amyloidogenic pathway was a pathological mis-metabolism of the protein. However, it has been found that small amounts of Aβ are secreted in normal, healthy individuals (reviewed in Selkoe 1994). Any compound or mechanism that inhibits the β (or

amyloidogenic) pathway is of obvious interest as it would reduce the amyloid burden in the brain. There is evidence that an increase in α-secretase processing leads to a decrease in Aβ production (Selkoe 1994). Consequently, there have been a number of studies investigating the intracellular regulation of APP processing. Some of these results are summarised in Figure 3.5.1 In 1987, Stokes and Hawthorn showed that the concentration of phosphoinositide (PI) was reduced in post-mortem AD brain (Stokes and Hawthorn 1987). Receptors coupled to the PI signalling pathway (Pacheco and Jope 1996) include the muscarinic and metabotropic glutamate receptors (mGluRs). Using a metabolically stable GTP analogue, GTPγS, it was shown that carbachol (a muscarinic agonist) produced lower PI breakdown in AD brains than in age-matched controls, suggesting an uncoupling of the receptor from its transduction mechanism (Ferrari-DiLeo and Flynn 1993). A decrease in GTPγS stimulation of adenylate cyclase has also been observed (Cowburn, O'Neill et al. 1992), and a reduction in $G_{i\alpha}$ levels (Ross, McLaughlin et al. 1993). IP_3 binding is also severely compromised (Young, Kish et al. 1988; Garlind, Cowburn et al. 1995).

Class I mGluRs (mGluR 1 and mGluR 5) are also linked to the PI system. Studies using drugs which target these receptors have shown that stimulating or blocking them can alter APP processing (Lee, Wurtman et al. 1995; Ulus and Wurtman 1996; Jolly-Tornetta, Gao et al. 1998). For example, *trans-(1S, 3R)*-1-amino-1, 3-cyclopentane dicarboxylic acid (ACPD), an agonist of group I and II mGluRs, results in an increased release of soluble APP (APP_s) in the systems above. The selective antagonist of mGluRs, (±)-α-methyl-4-carboxyphenylglycine (MCPG), blocks this increase in APP_s. In one study (Jolly-Tornetta, Gao et al. 1998), it was observed that IP_3 accumulated after treatment with ACPD as well as the increase in APP_s consistent with stimulation of the receptors producing a stimulation of the corresponding signal transduction pathway.

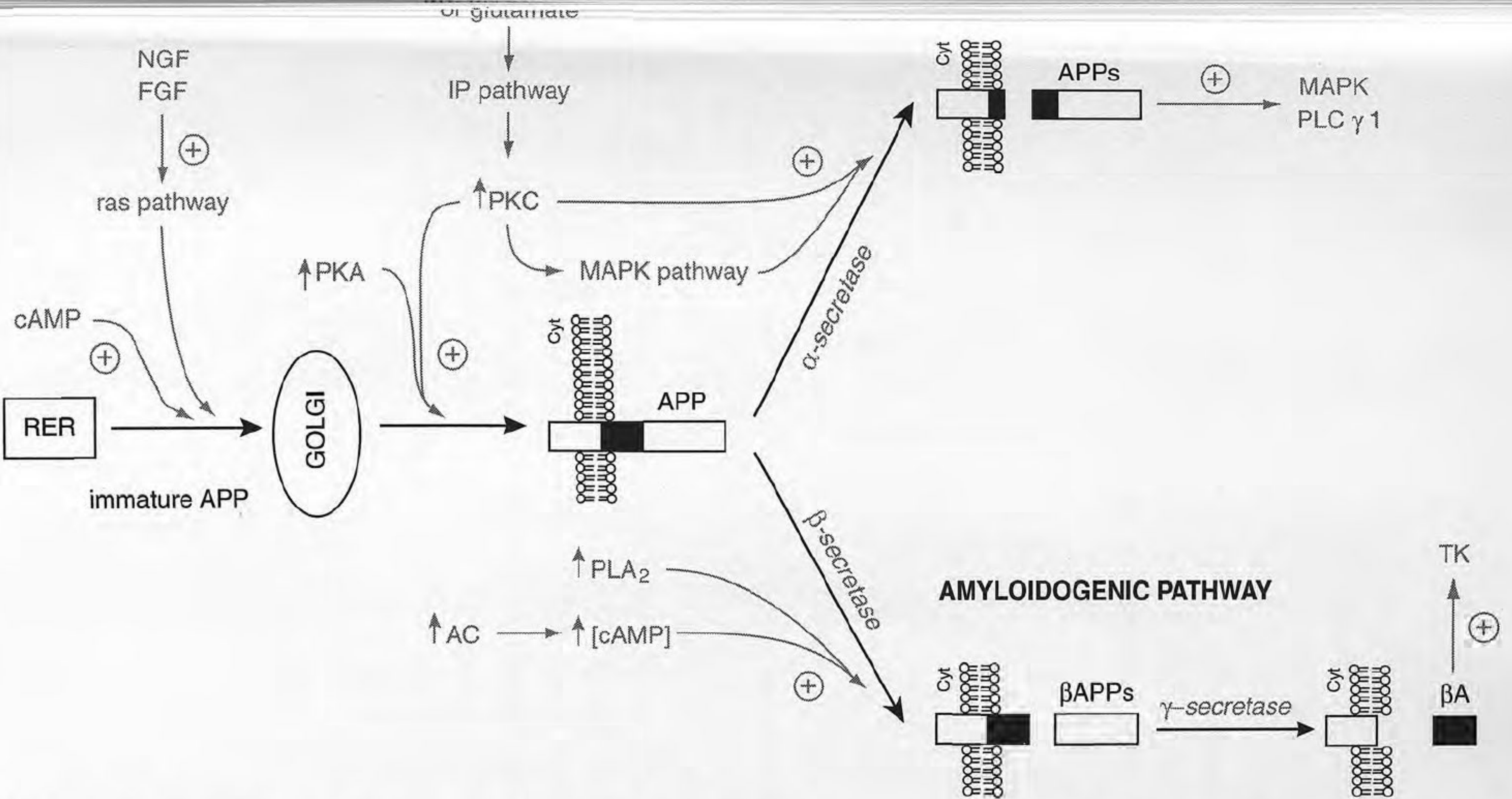


Figure 3.5.1: The effects of second messengers on APP metabolism. The synthesis and metabolism of APP is shown, greatly simplified. After synthesis on the RER and transportation through the Golgi, the mature protein is cleaved either by the α–secretase or the β and γ secretases. The actions of different molecules at different stages are shown. The products APPs and Aβ can also affect enzymes in intracellular cascades.

Abbreviations: AC - adenylate cyclase; cAMP - cylic adenosine monophosphate; Cyt - cytosolic; FGF - fibroblast growth factor; IP - phosphoinositide; m1, m3 - muscarinic receptors; mGluR - metabotropic glutamte recpetor; MAPK - mitogen activated protein kinase; PKA - protein kinase A; PKC - protein kinase C; PLA_2 - phospholipase A_2; PLC - phospholipase C; NGF - nerve growth factor; RER - rough endoplasmic reticulum; TK - tyrosine kinases; ↑ - an increase, activation or upregulation; ⊕ - stimulates pathway.

Protein kinase C (PKC), an enzyme linked to learning and memory processes, has also been found at reduced levels in AD brain (Shimohama, Narita et al. 1993), and at both reduced levels and activity level in aged rat brain (Battaini, Del Vasco et al. 1990). In post mortem AD brain, reduced binding of phorbol esters (activators of PKC) has been found, suggesting a decreased level of activity (Cole, Dobkins et al. 1988; Shimohama, Narita et al. 1993). However, these results have to be interpreted with caution as the methods used cannot distinguish different isoforms of PKC which may be differentially regulated in AD.

Several lines of evidence suggest that PKC can modulate APP processing. Treatment of cell lines with phorbol esters have shown that they produce significantly more APP_s (Buxbaum, Ruefly et al. 1994; Dyrks, Monning et al. 1994; Efthimiopoulos, Felsenstein et al. 1994) and, in some cases, a corresponding decrease in βA production. Consistent with these results was the finding that the PKC inhibitor H7 partially reversed the phorbol ester induced increase in secretion of APP_s (Slack, Nitsch et al. 1993). However, chronic over stimulation of PKC by phorbol esters has been found to have the opposite effect. High doses and prolonged treatment by phorbol esters have been shown not only to overactivate PKC but also to translocate it to the cell membrane and eventually to downregulate it (Matthies, Palfrey et al. 1987; Stabel, Rodriguez-Pena et al. 1987). Using phorbol 12-myristate 13-actetate (PMA) at very high concentrations it was found that human cortical neurons degenerated over a period of 3 to 24 hours and caused an increase in two AD markers, Alz-50 and 5E2 staining (Mattson 1991). Pathological effects have been found with multiple injections of PMA into the rat neocortex (Masliah, Mallory et al. 1993). It is possible that in AD excessive amounts of growth factors, cytokines or their receptors may result in hyperactivation of signal transduction pathways and induce cell death (Horsburgh and Saitoh 1994).

Intracellular cAMP levels mediate a wide variety of extracellular signals such as neurotransmitters, hormones and growth factors. The APP promoter has a number of regulatory elements, including a binding site for the AP-1 transcription factor. *c-fos*, a constituent of this complex, can be activated by cAMP responsive mechanisms. It has been shown that cAMP dependent regulation of APP mRNA upregulates APP mRNA in BT4C and BT4Cn glioma cells (Gegelashvili, Bock et al. 1996). (Efthimiopoulos, Punj et al. 1996) found that forskolin, an activator of adenylate cyclase (AC) resulted in an increased cellular concentration of cAMP and also inhibited the normal and phorbol-ester stimulated release of nexin II (the secreted portion of APP_{751} after α-secretase cleavage). This suggests that activation of the AC pathway inhibits APP_s secretion by regulating the levels of intracellular cAMP.

The mitogen-activated protein kinases (MAPK) ERK1 and ERK 2 represent a subgroup of kinases that play a role in hormonal signal transduction. They are growth-factor stimulated protein kinases that phosphorylate and thus modulate the characteristics of other proteins that have important regulatory functions. These include other protein kinases, transcription factors and cytoskeletal proteins such as tau. Abnormal activation of these kinases may enhance the phosphorylation of tau, thereby contributing to the pathology of AD. ERK 2 has been found in plaques and tangles from AD tissue (Trojanowski, Mawal-Dewan et al. 1993).

MAP kinases have also been found to be involved in the regulation of APP metabolism (Mills, Charest et al. 1997). Using two different approaches, it was found that activation of this pathway is necessary for regulation of secretion of APP_s. Using a pharmacological block of mitogen-activated protein kinase kinase (ERK 2), the only known target of ERK 1, they found that ERK 1 activation was inhibited and that NGF and phorbol ester stimulation of APP_s was inhibited. They also overexpressed mutant ERK 2

in HEK 293 cells. The mutant protein has a single amino acid change at its ATP-binding site, rendering it inactive. In cells expressing this mutant protein, again it was found that the phorbol ester induced increase of ERK 1 phosphorylation was inhibited and phorbol ester stimulation of APP_s secretion was also antagonised. These results imply that the PKC stimulation of APP_s release is mediated through the MAPK pathway.

4. Animal Models.

4.1 Introduction.

Models are important in any field, and AD is no exception. A clear understanding of the cause, onset and progression of the disease has not been resolved. Using animal models, one can test specific predictions, gain an insight into basic mechanisms and verify *in vitro* studies. As research progresses, a good model also allows compounds with putative beneficial effects to be tested. Even a good partial model of the disease can still be useful, as long as the results are interpreted in the correct context.

4.2 Nonhuman Primate Models.

Nonhuman primates also develop pathology and behavioural abnormalities similar to those found in AD. One of the best models is the species *Macaca mulatta*, which can live beyond 35 years of age. Cognitive and memory deficits appear in the second decade and become more prevalent as the animal ages. Older monkeys show amyloid deposition (Struble, Price et al. 1985; Selkoe, Bell et al. 1987), dystrophic neurites (Cork, Masters et al. 1990; Martin, Sisodia et al. 1991) and depletion in neurotransmitter systems (Goldman-Rakic and Brown 1981; Beal, Walker et al. 1991), although these features are less severe than those seen in human subjects with AD (Struble, Price et al. 1985; Cork, Masters et al. 1990). More specifically, phosphorylated neurofilaments, Aβ immunoreactivity, microgliosis and astrocytosis are also seen (Martin, Sisodia et al. 1991; Wisniewski and Weigel 1991).

4.3 Rodent Models.

Unlike other species, such as dogs, primates and bears, these animals do not develop amyloid deposits with ageing. Thus, some intervention is required. Early models relied on lesions or drug treatments in an attempt to mimic the effects of the disease. More recently, the development of transgenic technology, along with the identification of genes implicated in AD has led to more accurate (although still incomplete) transgenic models of the disease.

4.4 Lesion Models.

Several groups have attempted to create animal models for the behavioural impairments found in AD by selectively lesion brain areas known to degenerate in AD brain. Many attempts focused on the septal region as a result of reports that large and relatively selective cholinergic loss in the basal forebrain were found in the disease (Bartus, Dean et al. 1982; Coyle, Price et al. 1983; Agid, Ruberg et al. 1987). Behavioural impairments are produced by lesioning septal or cholinergic neurons, and the result of the loss of specific cell populations can be determined (Bartus, Dean et al. 1982). However, this type of model has some major disadvantages: neurodegeneration in AD is widespread and involves more than one neurotransmitter system, even with more specific toxins which offered the opportunity to study the consequences of particular cell loss, no information was forthcoming about the fundamental causes of AD and such lesions did not satisfactorily reproduce the pathology seen in the disease (Dahl, Bignami et al. 1980; Wisniewski, Sturman et al. 1982). However, with the advent of more relevant toxins, this approach may be revived and applied to certain areas of the disease. For example, infusions of okadaic acid have been reported to produce elevated phosphorylated tau staining and extracellular Aβ immunoreactive lesions (Arendt, Holzer et al. 1995).

4.5 Infusion Models.

Direct infusion or injection of Aβ has been carried out to establish a model of AD. Generally, small injections of nanomolar concentrations of Aβ do not cause any short term neurotoxicity. Problems occur with attempting to inject larger amounts (in the microgram range) as the Aβ tends to aggregate before it is injected and the solvents used to prevent this are themselves neurotoxic. An initial report (Kowall, Beal et al. 1991) using 10-15μg of Aβ found some neurotoxicity. This result was replicated by another group (Waite, Cole et al. 1992). However, they also found that the toxicity varied with the solvent used for the Aβ. Another disadvantage with this method is the damage the injection needle or infusion cannula causes and the limited diffusion of Aβ away from the injection site; only localised toxicity is seen.

4.6 Current Models.

The development of transgenic technology has allowed a new approach to modelling the disease. Generally, the APP gene, with mutations found in human populations, is coupled to a suitable promoter and is expressed in the animal. Doing so has resulted in animals displaying to neuropathology which is more similar to that seen in AD (although still not identical -- for example, the models lack neurofibrillary tangles). The major advantage of this approach is that the pathology develops over time, with young animals having apparently normal brains but building up an accumulation of Aβ and plaques as they age. A selection of these models is discussed in the following section.

4.7 Transgenic Models.

4.71 The PDAPP Mouse.

This model overexpresses mutant human APP and has AD-like neuropathology (Games, Adams et al. 1995), (Masliah, Sisk et al. 1996), (Irizarry, Soriano et al. 1997). A platelet derived growth factor (PDGF) β-chain promoter drives a construct containing a mutant human APP minigene. The mutation is a single amino acid change at position 717 (isoform 770 numbering) where valine (V) is substituted by phenylalanine (F). This mutation is associated with FAD (Murrell, Farlow et al. 1991). Introns 6-8 are included in the construct which allows alternative splicing of exons 7 and 8. This results in all 3 isoforms of hAPP being expressed in the brain (Games, Adams et al. 1995). The choice of promoter and the inclusion of the introns in the construct means that very high levels of APP and Aβ are detectable in the mouse brain; greater than 10-fold higher levels are seen compared to endogenous levels of mouse APP or levels of Aβ seen in human AD brains. The genetic background of these mice is mixed C57Bl/6, DBA and Swiss-Webster strains.

These animals exhibit an age-dependent pathology, something which previous animal models had failed to show. Between the age of 4-6 months, no obvious pathology was seen. However, between approximately 6-9 months, the transgenic animals begin to show deposits of human Aβ in the hippocampus, corpus callosum and cerebral cortex. These increase with age, the deposits becoming more and more dense as the animals pass 9 months of age. Between the ages of 12 and 18 months, a marked increase in Aβ deposition is observed. As the animals pass 18 months, the level of accumulated Aβ is higher than that seen in AD (Hyman, Irizarry et al. 1997; Irizarry, Soriano et al. 1997). At this age, the dentate gyrus is completely

covered in deposits of Aβ. By 24 months, the entire hippocampus and cortex is covered in such deposits.

The nature of the plaques is similar to that of AD. Neuritic plaques were seen (amyloid cores accompanied by extensive clusters of dystrophic neurites) as were dystrophic neurites without an amyloid core (Masliah, Sisk et al. 1996). Other important features seen in this model are astrocytosis and microgliosis (activated astrocytes and microglia surrounding the plaques) which are observed in human AD brain. These features are also age-dependent. At 6 and 8 months of age, no reactive astrocytes are seen in mouse brain. However, clusters begin to appear at 10 months and at 18 months, the entire hippocampus is filled with reactive astrocytes. A large increase in astrocytosis is seen after 12 months, i.e. astrocytosis lags the plaque formation by about 2 months.

Microgliosis follows the neuritic pathology more closely than the astrocytosis with clusters of reactive microglia appearing at 8 months of age. A large increase in number is seen after 8 months and this increases to 24 months, the oldest age tested, as yet. However, even at 24 months, no neuronal loss is observed in these animals (Hyman, Irizarry et al. 1997), although there is a decrease in the marker synaptophysin, indicating a decrease in synapses. (It must be noted, however, that young heterozygous PDAPP mice initially have 20% less synaptophysin than their control litter-mates (Hyman, Irizarry et al. 1997)).

To summarise, this rationale for this model is to take a mutation found in FAD and overexpress it in mouse brain. The mice show the appropriate pathology (plaques and diffuse deposits of Aβ, astrocytosis and microgliosis) which is age-dependent. These are features of AD. Where this model is limited is that no PHF or tangles develop and no overt neuronal loss is seen, even at 24 months of age, although there does appear to be a decrease in the number of synapses. The very nature of the model is somewhat artificial; there is vast overexpression of both mutant hAPP and Aβ at levels not seen in AD. These mice express the non-neuronal isoforms of APP in the brain (APP_{751} and APP_{770}). It may be argued that this system represents a good model of plaque deposition rather than a model of the whole disease. Nevertheless, as plaque deposition in AD always occurs and may possibly be a central event (Selkoe 1994), it will continue to be useful, expanding the understanding of plaque formation and providing a model to test putative Aβ inhibiting compounds.

4.72 The Tg2576 Mouse.

In 1996, Hsaio et al published pathology and behaviour of another mouse model, referred to as the Tg2576 mouse (Hsiao, Chapman et al. 1996). Again, the starting point was a mutation found in FAD, the "Swedish mutation". This is a double mutation resulting in $lysine_{670}$ converted to aspartate, and $methionine_{672}$ to leucine (APP_{770} numbering). The $hAPP_{695}$ (the neuronal type) was driven by a hamster prion protein (PrP). The genetic background was C57B6/SJL F_2 mice backcrossed to C57B6.

This line produces a five-fold increase in the concentration of Aβ(1-40) and a 14-fold increase of Aβ(1-42/43) between the ages of 2 to 8 months (designated young animals) and 11 to 13 months (old animals). Plaques were seen in mice with elevated Aβ levels in a similar pattern to the PDAPP mouse. Again, microgliosis and astrocytosis surrounded the plaques, consistent with AD.

Hsiao et al also performed two behavioural studies. The first was spatial alternation in the Y-maze tested at 3 and 10 months of age. In the second, three groups of 9 to 13 month old transgenic mice and 10 to 14 month old controls underwent a spatial reference memory task in the watermaze (Morris 1984, see also methods section) at 3, 6 and 9 months of age.

Young transgenic mice showed no difference in the amount of spontaneous alternation compared to controls. However, at 10 months of age, transgenic mice showed less tendency to alternate. This reduced alternation is characteristic of animals with hippocampal damage.

In the watermaze, they found that 9 to 10 month old transgenic mice were also impaired relative to age matched controls. With respect to latency data, transgenic mice aged either 2 months did not differ from controls at any point during training. 6 month old transgenic mice only differed from controls on the last day of training whereas the 9 to 10 month group differed from control mice on all trials. At the end of training, animals were given a transfer test where they were allowed to swim for 60s with the platform absent. This provides another measure of spatial learning and memory. The percentage of the trial spent in the quadrant where the platform had been located was analysed, as well as the number of times the animals crossed the position where the platform had been and the total platform crossings (with 4 'imaginary' platforms at the centre of each quadrant). 9-10 month old transgenic mice spent less time in the training quadrant with

respect to control animals. No difference was seen between the groups at 2 or 6 months of age. Old transgenic mice also crossed the platform's location less than controls and younger transgenic mice.

9 to 10 month old animals were also subjected to visible platform training where the platform is marked by a single cue, all other extra-maze cues being invisible. No difference in latency was seen on the first day but transgenic animals were impaired on the subsequent 3 days, and this difference was significant on days 2 and 4.

The performance of the control mice at 9 months of age in the transfer test was greater than that at 2 and 6 months and this may confound the result (2 month old controls spent approximately 39% of the trial in the training quadrant, 6 month controls spent approximately 35% of the time in this quadrant and 9 month old animals spent about 47%). However, data from platform crossings shows that there is no difference the total number of platform crossings but old transgenic mice cross the platform's position less frequently than controls or younger transgenic animals.

The significant difference between groups on the visible platform test suggests a possible sensorimotor deficit, but both groups were equivalent on the first day of training showing that naïve transgenics can perform as well as controls at 9-10 months. This may result from the increasing number of plaques in the older animals, although no change in transfer test performance was seen between 9-10 month old transgenics and the younger transgenics.

This study demonstrates that these animals develop senile plaques, astrocytosis and microgliosis consistent with AD. These animals can still learn, although they are impaired relative to age-matched controls. Whether this impairment is age-dependent is somewhat more ambiguous. Further investigation of these mice has lead to the finding that aged (19

month old) transgenic mice show significantly less long-term potentiation (LTP) in the CA1 region of the hippocampus than controls, and show a complete block of LTP in the dentate gyrus (Chapman, White et al. 1998). The amount of LTP in the transgenic animals also correlated with performance on a forced-choice alternation task in the T-maze, providing evidence for an age-dependent deficit and that synaptic plasticity is affected in AD.

4.73 Carboxy-terminus APP Transgenic Mice.

Another approach to modelling AD was demonstrated by Nalbantoglu et al in 1997 (Nalbantoglu, Tirado-Santiago et al. 1997). They generated mice with a construct containing APP cDNA encoding residues 591-695 (APP_{695} numbering), which spans the Aβ domain and the carboxy terminus, cloned into the first exon of the human neurofilament NF-L gene and under transcriptional control of its regulatory elements.

With ageing, the mice showed increased extracellular Aβ immunoreactivity, as shown by immunohistochemistry (staining using antibody R1282), which presented as dense, compact structures in the cortex and hippocampus. No such staining was seen in control animals. This reactivity was accompanied by a generalised gliosis which appeared at about 4 - 5 months of age. A change in microglial reactivity was also seen. Cell counting was undertaken in CA1 and revealed a decrease in both cell number and density in the transgenic animals.

Searching for a phenotype, this group also performed a watermaze study. 8 month old animals transgenic for the C- terminal fragment of APP or for the gene chloramphenicol acetyltransferase driven by the same *NF-L* sequences were tested. A pre-training phase was given to ensure the animals could swim and climb onto the platform. After this, mice were given 8 trials a day for 7 days. The platform was moved to the opposite quadrant and mice were again tested for 7 days (8 trials per day). Following this, visible platform training was given for the next 7 days where the location of the platform was moved between days. Finally, another 7 days (8 trials/day) of the hidden platform task was given.

The group transgenic for the C-terminal fragment took longer to find the platform than the other group on all 3 phases of hidden platform phases. The control group found the platform on more occasions than the 120s time limit set for each trial. Overall, the performance of the groups on the visible platform phase was equivalent, with no differences between the groups over the last four days of this task. Analysis of the transfer test revealed a spatial bias in the control group towards the platform's location but not in the transgenic group.

Transgenic mice swam faster than controls but swam over longer distances than the controls. The authors present figures of swim paths for both groups at different stages of the experiment. These suggest that the transgenics are more thigmotaxic than controls, which may account for the longer path lengths.

The mice were examined for any difference in long-term potentiation (LTP) as this is a model system for the basis of learning and memory. Synaptic potentiation was induced by theta-burst stimulation (TBS) and followed for 60mins in the CA1 area of hippocampal slices. No difference was seen between the groups 2 minutes after tetanisation, but thereafter there was a progressive decay of LTP in the transgenic group, with a significant reduction seen after 10 minutes. No significant difference was seen between the groups when paired-pulse facilitation was examined. Long-term depression was also studied, but no difference was seen.

A binding study was undertaken to determine whether impairment in LTP was due to a reduction in NMDA receptor number or function. The binding of 100nM ^{3}H-glutamate was assayed and no difference was seen in the amount of binding in various regions of the brain including CA1. The function of the receptor was studied by measuring the NMDA response evoked in the presence of a low Mg^{2+} concentration and DNQX (the AMPA antagonist). It was normal. This suggests that a decrease in NMDA receptor function alone is unlikely to account for the impairment in LTP.

The authors have presented an impairment in spatial learning and a decrease in LTP. However, it appears that the transgenic animals were much more thigmotaxic than controls and this would account for the longer escape latencies. They were not thigmotaxic on visible platform trials, but they may have adopted a different strategy for this phase of the experiment. In addition, the cues were not the normal extra-maze type used. They hung inside the maze 3cm above the water surface at the quadrant intersections. The link between reduced LTP and spatial learning is not straightforward; the impairment in LTP may be the result of non-specific effects of the transgene.

4.74 The SciosNova Mouse.

This group expressed full length APP_{695} and APP_{751} cDNAs in the JU mouse under the control of a rat neural-specific enolase promoter (Quon, Wang et al. 1991). A subsequent publication using semiquantitative PCR showed that transgene APP mRNA was expressed at higher levels than endogenous APP mRNA (Higgins, Rodems et al. 1995). However, this study has been criticised as the primers used to amplify both the endogenous and transgenic APP contained a bas mis-match relative to the endogenous mouse mRNA, possibly resulting in an underestimation of the amount of endogenous mRNA produced (Greenberg, Savage et al. 1996). Immunoblot analysis of protein extracts from the APP_{751} mice showed elevated levels of APP (Quon, Wang et al. 1991). This may be difficult to interpret, however, as mice have been shown to produce mainly APP_{695} mRNA and protein in their brains (Ohyagi, Takahashi et al. 1990; Buxbaum, Christensen et al. 1993). If the transgene was overexpressed, the immunblot should have shown a higher molecular weight band. This was not observed.

Immunhistological studies using antibodies against Aβ and APP revealed enhanced staining in neurons throughout the brain but particularly in regions surrounding CA1 to CA3 of the hippocampus. Diffuse extracellular deposits were seen using antibodies which recognised Aβ 1-28 and the carboxy terminus of Aβ 1-42 (Higgins, Holtzman et al. 1994). Histology was carried out on mice 2-3 months old and mice which were 22 months old. In young animals, 27% of sections from the APP_{751} mice had at least one deposit, compared to 1% of sections from APP_{659} mice and 5% of controls. In old APP_{751} mice, 42% of sections stained positively for the deposits. These sections also stained for Alz 50, which recognises abnormally phosphorylated tau protein. No data were presented for old controls or old APP_{695} mice. No evidence was found for synaptic or neuronal loss.

In another study with these animals, an age-related spatial learning and working memory deficit was reported in APP_{751} mice (Moran, Higgins et al. 1995). However, in the watermaze, these mice were also impaired on visible platform training indicating that at least some of this deficit is due to non-cognitive factors.

4.75 APP 14, APP 22 and APP 23 mice.

Sturchler-Pierrat et al (Sturchler-Pierrat, Abramowski et al. 1997) generated three transgenic models, again using mutations found in FAD. The APP 14 line used a human Thy-1 promoter to drive human APP_{751} containing the Swedish mutation (the same as that used in Hsaio et al). The APP 22 line was identical to APP 14 except it carried an additional mutation, called the London mutation, where a I at residue 717 (isoform 751 numbering) is substituted for a the normal valine. Finally, the APP 23 line contained APP with the Swedish mutation driven by a murine Thy-1.2 gene. APP 14 mice showed no neuropathology up to the age of 2 years and will not be discussed further.

APP 22 mice show transgene mRNA in the throughout the brain, with high levels of expression in the hippocampus and neocortex. The transgene mRNA exceeds the endogenous mRNA by 2-fold. No Aβ deposits are seen in 12 month old transgenic mice, but by 18 months of age, plaques are detected in the hippocampus and neocortex. They are mainly of the diffuse type, although some neuritic plaques were detected. Plaque associated proteins such as heparan sulphate proteoglycan and inflammatory responses (indicated by GFAP, MAC-1, MHC class II and complement C3 staining) are also associated with deposits, consistent with the pattern seen in human post-mortem AD brains.

The pattern of mRNA distribution in APP 23 mice is similar to that of the previous line; however, the transgene is 7-fold overexpressed. This line first shows Aβ deposits at 6 months of age which increase in size and number until by 24 months they occupy a large proportion of the hippocampus and neocortex. At this age, plaques are also seen in the thalamus, olfactory bulb and the caudate putamen. Consistent with higher expression in this line, the deposits stain positively with Congo-red, indicating that they are dense-core or neuritic plaques. Even at 6 months old, most of the plaques are neuritic. The same inflammatory markers and plaque associated proteins are seen in this line as in APP 23. Immunostaining with an antibody specific to Aβ 1-42 appears similar in intensity to that in the APP 22 line despite the larger overexpression. This is due to the London mutation which has been found to produce more Aβ 1-42 (Suzuki, Cheung et al. 1994), the more amyloidogenic species of Aβ.

In both lines, dystrophic neurites are seen round the outer margin of neuritic plaques. A reduction of cell bodies adjacent to the plaques is seen in areas with high cell density, such as the hippocampal pyramidal cells. This may be cell displacement, but the authors argue that it could be cell loss as there is no compression of the intracellular space. The neuritic plaques show strong acetylcholinesterase staining and a local distortion of the cholinergic fibre network is seen both in the plaques and surrounding their periphery, again consistent with AD brain.

These lines also show some evidence for neurofibrillary pathology. Although no NFTs were detected using the Gallyas method, immunostaining with antibody AT8, which recognises phosphoserine residue 202 on tau, was found only associated with Congo-Red positive plaques. The staining pattern suggested distorted neurites surrounding the cores of the deposits. Similar results were obtained with antibodies PHF1, R27 and R32, which all recognise different phosphoepitopes of tau. In line

APP 23, increases in phosphorylation of tau are seen at 6 months and 15 months using antibodies AT8 and R27. Age-matched littermate controls also show some immunoreactivity, but this is much weaker and does not increase with age. A phosphorylation - independent tau antibody, tau7 showed that these results could not be explained by an up-regulation of the protein in transgenic brains.

In summary, two lines described by this group show Alzheimer-like pathology. This is different in each line, which may offer the opportunity of to study plaque maturation processes. The appearance of hyperphosphorylated tau is tantalising, however, no tangles were observed, although this needs to be confirmed ultrastructurally. Behavioural analyses of these mice would be interesting, and are no doubt underway. Based on other studies, one would expect mice to be impaired on spatial memory tasks, although the specificity of such deficits would have to be confirmed. It is possible that the two lines may exhibit different performance on the same behavioural task at different ages, if the task were sensitive enough.

4.76 Summary.

Transgenic models, of which the above represent only a selection, are perhaps amongst the best developed yet. Although it is true that aged nonhuman primates have the advantage of closer similarity to humans, rodents offer an opportunity to understand more basic mechanisms of the disease. However, the transgenic models are not without their disadvantages. Background strain differences make it difficult to compare models, particularly at the behavioural level. Different promoters are used, which results in different magnitudes of overexpression of Aβ. These models also express different isoforms of Aβ whereas only APP_{695} is expressed in neurons. They also express endogenous murine APP as well as mutant human APP. While the pathology is undoubtedly encouraging, it is difficult to interpret if the behavioural deficits seen are purely down to these effects, particularly in the early stages. It is possible that the mere

overexpression of the transgenes has an effect before any overt neuropathology is seen. No transgenic model that exhibits plaques and tangles has been reported so far. However, the models currently available will be useful in understanding the mechanisms of plaque deposition and provide strong evidence that Aβ deposition is central to the disease process.

5. Behavioural Experiment.

5.1 Introduction.

The purpose of this study was to characterise the behavioural features of a possible mouse model of Alzheimer's disease. The model under investigation, the PDAPP mouse has been described previously (see above and Games, Adams et al. 1995). Briefly, this mouse overexpresses human APP containing the mutation V717F which is associated with FAD. The initial report showed aspects of appropriate neuropathology and this had been confirmed in subsequent reports (Masliah, Sisk et al. 1996; Irizarry, Soriano et al. 1997; Johnson-Wood, Lee et al. 1997).

One of the first regions of the brain to be affected by AD is the temporal lobe, particularly the entorhinal cortex and then the hippocampus. One task sensitive to hippocampal damage is the watermaze (Morris, Garrud et al. 1982). It provides a measure of spatial memory. Since AD has an insidious onset and progresses with age, a protocol was designed that enabled individual mice to be tested repeatedly at different ages. This allowed a comparison between performance at young and old ages as the pathology developed. In principle, it could also reveal correlations between pathology and behavioural deficits if any were present, but serial sacrifice was not included because of the numbers of animals that would be required to achieve statistically reliable results

5.2 General Methods

5.20 Subjects.

60 transgenic mice on a C57Bl/6, DBA and Swiss Webster background were shipped from Athena Neurosciences, USA. 30 contained the transgene and 30 were wild-type littermates. Mice were housed either individually or in small groups and given *ad libitium* access to food and water. The animal room was kept at a constant temperature of 25°C and on a 12:12 light dark cycle.

During the study, some of the animals died. Table 5.20.1 gives the number of animals alive at each stage of training. Statistical analyses were carried out only on those animals that reached the end of the study.

AGE OF MICE	TRAINING SESSION	PDAPP N	CONTROL N
4 months	Cue Task	26	25
4-5 months	DMP Pre-training	24	24
6-7 months	Series 1	23	24
8-9 months	Series 2	20	23
10-11 months	Series 3	19	23
12-13 months	Series 4	19	23
19-20 months	Series 5	17	18
20 months	Cue Task	17	18

Table 5.20.1: Mortality throughout the study.

5.21 The Watermaze.

Behavioural testing was carried out in an open-field watermaze (Figure 5.21.1) as described in Morris (1984). Briefly, the watermaze is a cylindrical pool, 2m in diameter and filled with water at a temperature of 25 ± 1°C. The water is made opaque by the addition of 600mls of latex (Cementone-Beaver Ltd, UK). The only means of escape for the mouse was via a 30cm diameter platform submerged 1cm below the surface of the water. The watermaze is situated in a laboratory containing prominent extra-maze cues such as posters, primate caging and cupboards. Since the animal cannot see or smell the platform, it has to learn its position using these cues. This spatial learning task is hippocampal dependent, at least in rats (Morris, Garrud et al. 1982).

The following sequence of events constitutes a trial. The mouse is placed in the water at one of four start points (designated north, south, east or west) and released. The mouse swims until it has found the platform or until 90s has elapsed, in which case it is guided there by hand. Once on the platform, the mouse remains there for 30s before being removed, dried and placed back into a drying cage. This cage is placed under an infra-red heat lamp for 10mins. Each animal receives 4 trials a day. The inter-trial interval of 10mins under the heat lamp helps to ensure that the mouse does not become fatigued or that its body temperature drops significantly, both of which would affect performance. The genotype of the mice (Tg+ or Tg-)was unknown to the experimenter throughout the duration of the study. Data was collected using an image analyser (HVS Image, Cambridge, UK) and analysed online by an RM 486 PC running software (Watermaze, UK) which sampled the co-ordinates at 10Hz. Data was stored for off-line analysis.

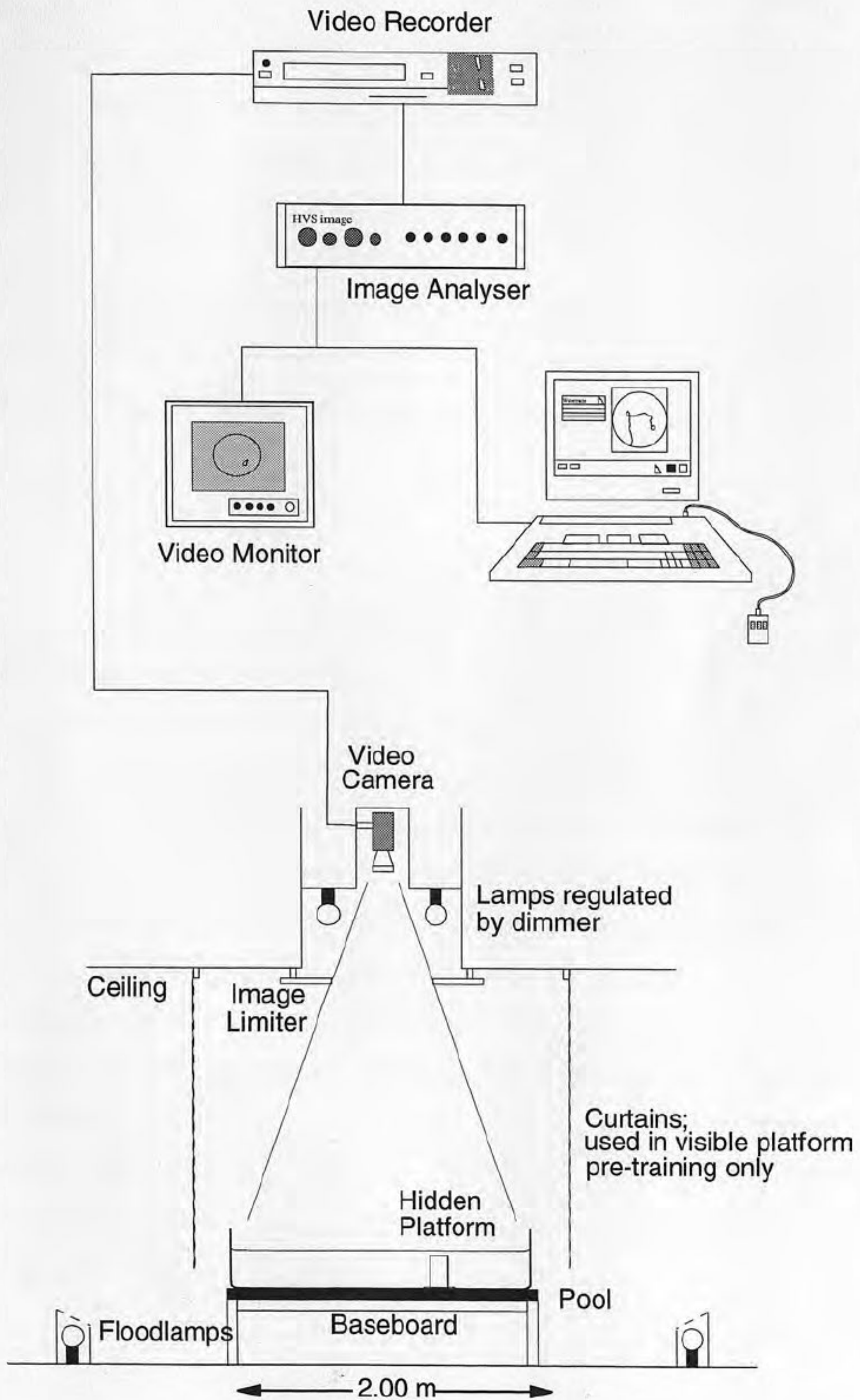


Fig. 5.21.1: The watermaze

5.22 Cue Training.

Each animal received 4 trials per day. Curtains were drawn round the pool to occlude the extra-maze cues. The platform, submerged 1cm below the surface of the water was rendered visible by attaching a red and white striped flag which protruded 10cm above the water surface. The purpose of this phase was, initially, to habituate the mice to the watermaze and then to ensure they could see, swim and climb onto the platform in a normal fashion. This task was repeated later (see experimental design) to check whether any sensorimotor defects had appeared with age.

5.23 Delayed Matching to Place.

The delayed matching to place (DMP) task formed the main body of the behavioural testing. Mice were given 4 trials a day with the platform hidden below the surface of the water and the extra-maze cues visible. Testing occurred over 10 days for individual series (5 days per week). On 8 of these days, the platform remained in a constant spatial location within each day, but moved position between days. This meant that the animal had to learn a new spatial location on each of these days. On trial 1, the mouse does not know the location, but on trials 2-4, the animal can 'match to place' i.e. has to return to the same location as on trial 1. This protocol was used because the longitudinal nature of the study involves repeated testing. If a more usual 'reference memory' task had been administered (one in which the platform remains in a constant location throughout training), practice effects may have contributed to the pattern of performance seen at successive ages.

The 2 remaining days of the 10 days per week were designated as variable location days These were counterbalanced across groups and series but the first always fell on day 4 or 5, and the second on day 9 or 10 of training. The procedure was identical to that for the DMP days except the platform also moved location on trial 2. Thus, the mice had an opportunity to learn a new platform position on trial 1 but this was no help to them on trial 2. Instead, they had, on trials 3 and 4, to match to the position on trial 2. This allowed an 'online' check to ascertain whether the animals were matching to place, i.e. that any reduction in latency between trials 1 and 2 in the DMP procedure reflects spatial memory.

As mentioned before, a longitudinal design was used (Figure 5.23.1). Testing began at 4/5 months of age. Cue training was given first for 5 days at 4 trials per day. Immediately after this, animals entered a period of pre-training where 10 days of DMP were given (age 5/6 months). Following this, at age 6/7 months the first series of training was given, consisting of 8 days of DMP training and 2 variable location days. This was repeated, at 2 month intervals, 4 times at month intervals followed by a final testing period 6 months later at age 19/20 months. Finally, another phase of cue testing was given at age 20 months.

5.24 Experimental Design.

Figure 5.24.1 shows the platform positions used in pre-training and the 5 series of DMP (including the variable location days). Positions were chosen that were not near the centre of the pool, to encourage mice to search the whole area of the pool, rather than swimming into the centre and bumping into the platform by chance. Before the experiment started, the mice were divided into 2 groups that contained equal numbers of PDAPP and wild-type mice (this was not done by the experimenter who remained 'blind' to the genotype of the animals). Two different sets of platform positions were used in each series, one for each group (Figure 5.24.1).

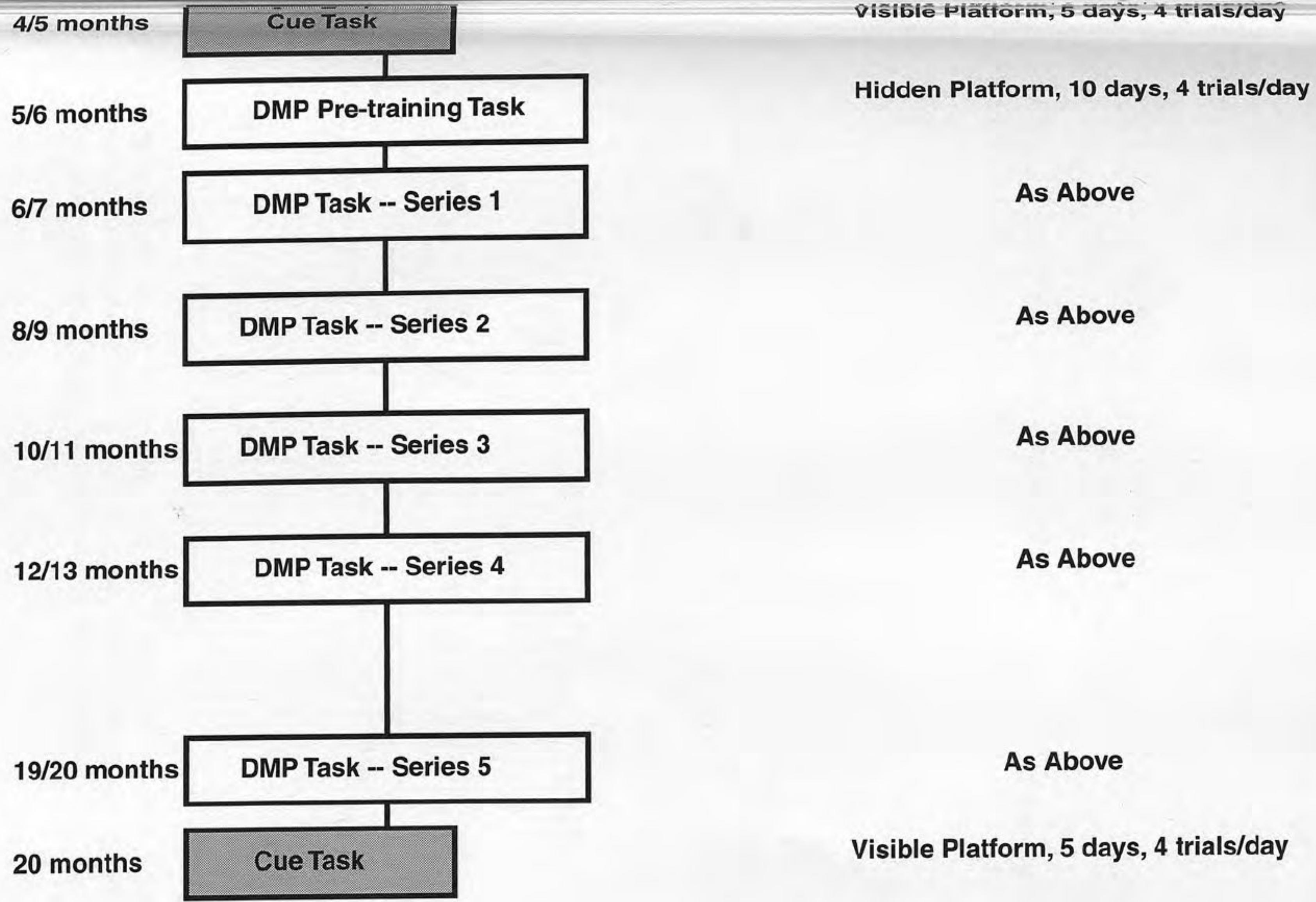
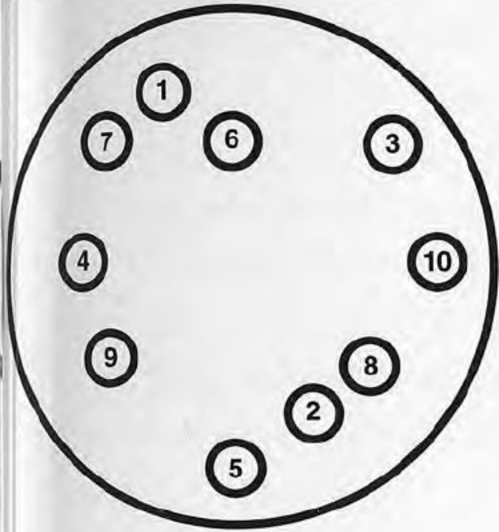
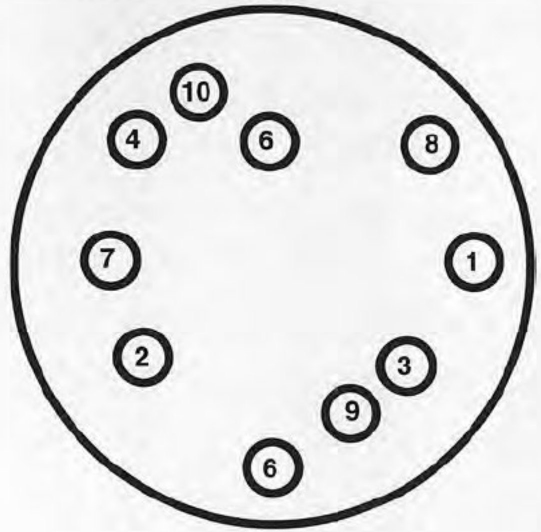


Figure 5.23.1: The design of the longitudinal study. Ages are given on the left, the experimental phase boxed in the centre, and experimental details on the right.

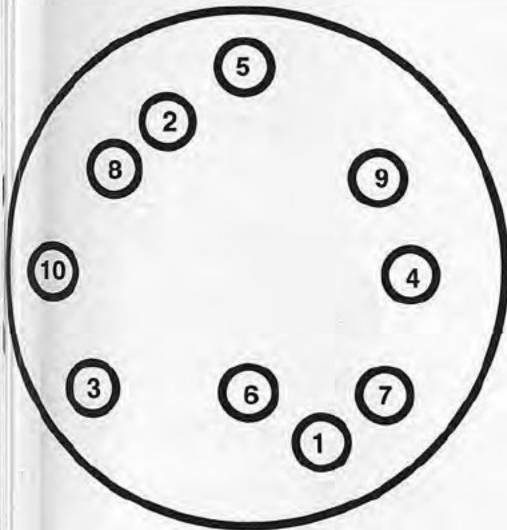
Sequence 1A



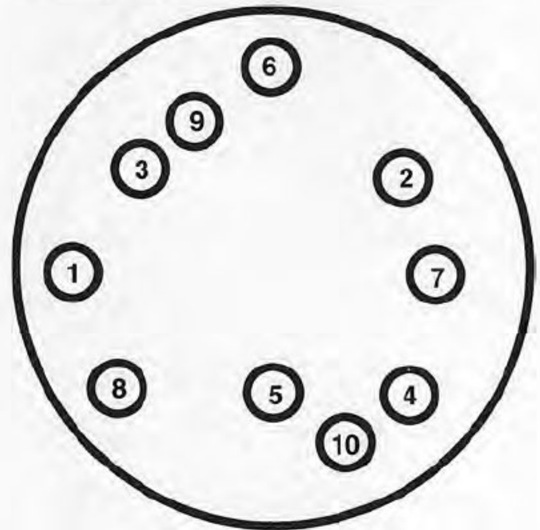
Sequence 1B



Sequence 2C



Sequence 2D



5.24.1: Platform positions used in the study. Half of the animals in the control group were assigned to sequence 1; the other half to sequence 2. The PDAPP group was also divided like this. Within each sequence group, the positions alternated between A and B for each series.

BIBL. UNIV. EDIN.

Within each group, 2 different sequences of platform positions were used (Figure 5.24.1). Both the sequence and platform positions alternated for a given group between each series. Furthermore, 2 sets of start positions were used, which were the reverse of each other. They were chosen so that the previous day's platform position was not between the start position for trial 1 and the new position that day. These also alternated between series. This design was chosen to avoid any practice effects over the long period of training, to ensure the task remained spatial.

5.25 Immunohistology.

At the end of the experiment, all mice were anaesthetised and perfused intracardially with 4% paraformaldehyde. Brains were removed and stored for a minimum of 48hrs in 4% paraformaldehyde. 40μm thick sections were cut on a vibratome and collected in an antifreeze solution (30% glycerol, 30% ethylene glycol and 40% 0.1M $NaPO_4$). They were stored at -20°C until stained.

Antibodies 3D6 and 8E5 (Athena Neurosciences) were used to detect different parts of the Aβ molecule. 3D6 recognises the first five amino acids of Aβ and requires a free amino terminus of Aβ (Johnson-Wood, Lee et al. 1997; Schenk, Masliah et al. 1997). Antibody 8E5 recognises APP 444-592 (isoform 695 numbering). Antibody 3D6 stains plaques with little background staining; whereas 8E5 recognises more diffuse amyloid deposits.

Sections were rinsed twice on a shaker at slow speed in phosphate buffered saline (PBS) for 10 mins each. Following this, sections were pre-treated with 1% Triton X-100 and 3% H_2O_2 in PBS for 20 mins. Sections were then washed twice in PBS as before. The primary antibody (3D6 or 8E5) was diluted 1:1000 in PBS with 1% horse serum. Sections were incubated with the primary antibody overnight at 4°C.

A Vectastain kit (Vector Laboratories, USA) was used in the first stage of identifying where the antibody had bound. Solutions were made according to manufacturer's instructions. Sections were washed twice with PBS as before and then incubated with the kit solution for 1 hr at room temperature. To stain the sections, a 3, 3'-diaminobenzidine (DAB) kit was used (Vector Laboratories, USA). DAB was also prepared according to the manufacturer's instructions. Four drops of the solution were added to each section. The reaction was controlled by inspection (the sections turn brown where antibody is present) and was stopped by placing the section into PBS.

Sections were mounted onto charged slides (to enhance adherence to the slide) in PBS and left to dry. They were counterstained with haematoxylin (which stains cell nuclei purple). Finally, coverslips were added with permount.

6. Results.

6.1 Behavioural Data.

Four main measures were used to analyse the behavioural data. These were latency (defined as the time taken to reach the hidden platform from the moment the mouse was placed in the watermaze), path length (the distance taken to reach the platform measured from the start position), the swim speed (the path length divided by the latency) and thigmotaxis (the percentage of time of the trial the animal spent swimming within 15cm of the side wall). Latency and path length are indexes of performance: an animal will search the whole pool if the platform position is unknown, but once this has been learned, it will swim towards the platform's location and so these measures will decrease; i.e. a decreasing latency and path length will give an indication of learning. Swim speed is analysed to ensure that differences in these first two measures are not due to sensorimotor defects. If an animal swims more slowly, the latency will be increased, even if has learned the position of the platform. Thigmotaxis is usually seen as another index of sensorimotor performance (Cain, Saucier et al. 1996), however, it may also be affected by cognitive factors: if an animal knows the platform location, it may spend less time near the side-wall as it moves away from them to swim towards the platform location.

6.2 Statistical Analyses.

Analysis of variance (ANOVA) was performed on each set of results. *Post-hoc* Newman-Keuls pairwise comparisons are given where appropriate. Uncorrected t-tests comparing different groups on individual days are also presented where appropriate.

6.3 Cue Training.

Figure 6.3.1 shows the latency data from the cue training at both ages tested (4/5 months and 20 months). Initially there was a mild deficit in the PDAPP group, but by the fifth day of training, asymptotic levels of performance had been reached and there was no difference between the groups. When the animals were re-tested at 20 months of age, the groups did not differ; all animals swam to the platform with a latency averaging less than 10 sec. An ANOVA revealed a significant impairment in the PDAPP group ($F = 10.40$, df 1/33, $p < 0.005$). Uncorrected t-tests showed the impairment was significant on days 1 and 2 of training, but not thereafter (Day 1: $F = 14.35$, df 1/326, $p < 0.001$; Day 2: $F = 10.90$, df 1/326, $p = 0.001$). Both groups improved across days ($F = 27.80$, df 9/297, $p < 0.0001$). Analysis of path length revealed the same pattern of results (Figure 6.3.2). A significant impairment was seen in the PDAPP group ($F = 4.48$, df 1/33; $p < 0.05$), although uncorrected t-tests showed that this impairment only occurred on the first two days of training (Day 1: $F = 6.20$, df 1/326, $p < 0.05$; Day 2: $F = 9.90$, df 1/326; $p < 0.005$). Both groups improved across days ($F = 24.2$, df 9/297, $p < 0.001$). No difference in swim speed between the groups was observed ($F < 1$, $p > 0.4$), (Figure 6.3.3) but PDAPP mice were more thigmotaxic than controls (Figure 6.3.4), ($F = 23.1$, df 1/33, $p < 0.001$). Uncorrected t-tests showed that the PDAPP group spent significantly more time near the side wall on the first 5 days of training (at age 4/5 months) but when re-tested at 20 months, no difference was seen. This was confirmed by a *post-hoc* Newman-Keuls pairwise comparison: the groups differed significantly ($p < 0.01$) over the first 5 days but not on the last 5 days.

To summarise, the PDAPP are impaired relative to controls on the first two days' exposure to the watermaze but reach an equivalent level of performance thereafter. All animals still alive at 20 months still perform well on this task (the latency data reveals they are at asymptote) so visual and sensorimotor effects are unlikely to

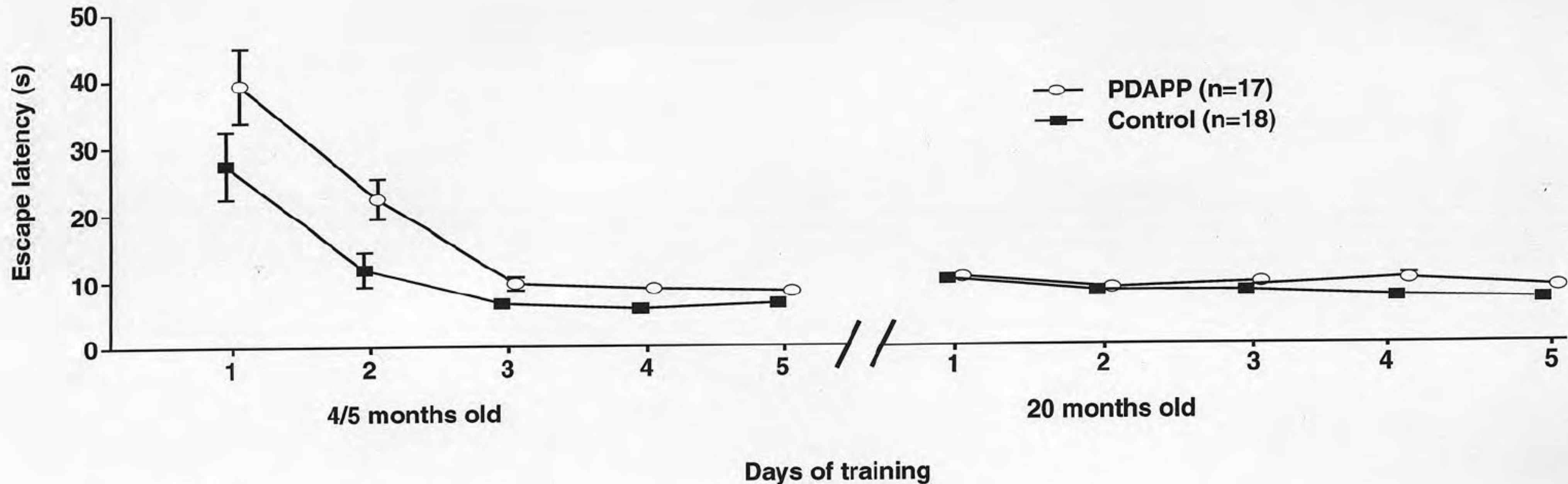


Figure 6.3.1: Time taken to reach the platform for both groups at both ages the cue task was administered. PDAPP mice are slower on the first two days, but thereafter show equivalent performance to controls.

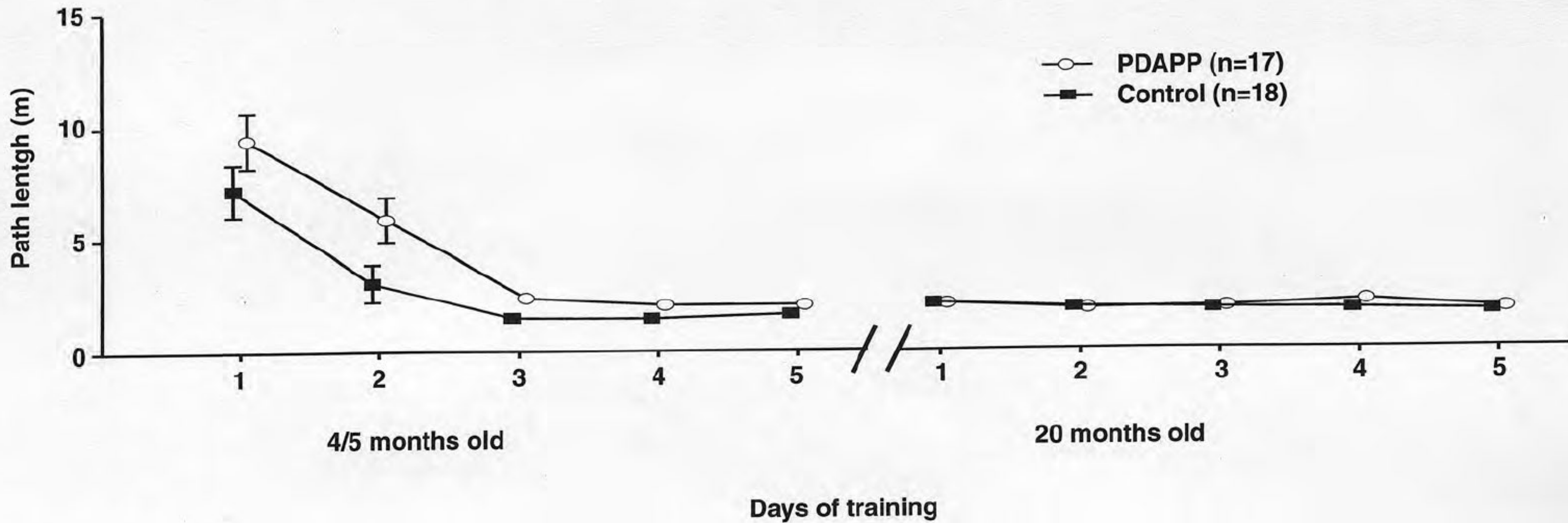


Figure 6.3.2: Distance taken to reach the platform for both groups at both ages the cue task was administered. The pattern mirrors that of the escape latency. PDAPP animals take longer paths on the first two days but are equivalent to controls thereafter.

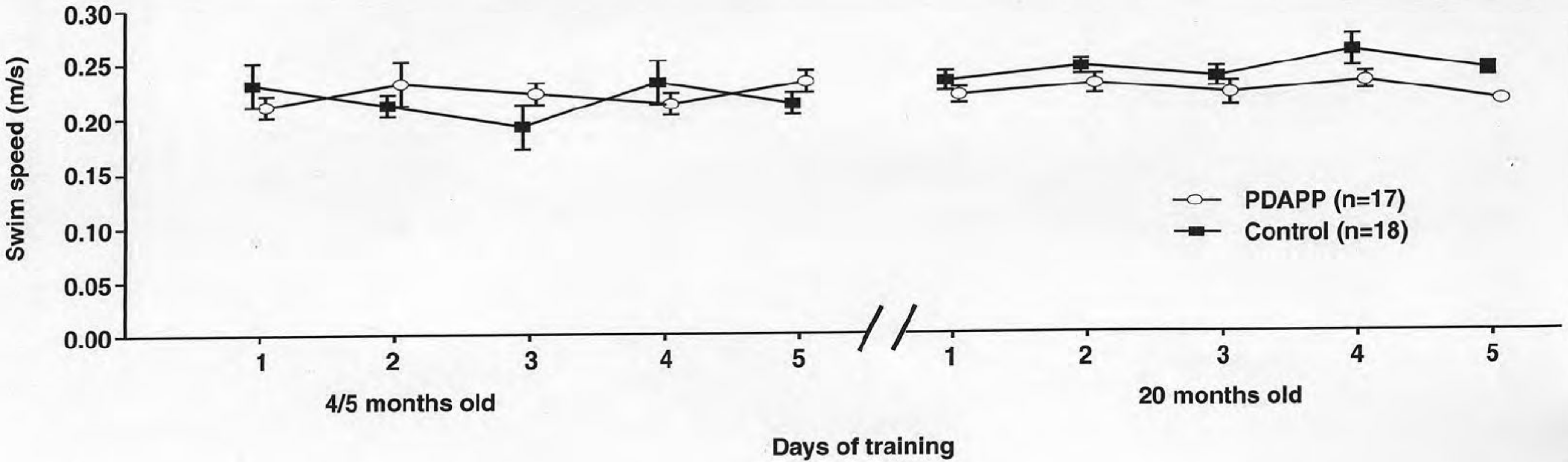


Figure 6.3.3: Swim speed for both groups at both ages they performed the cue task. There is no difference between the groups.

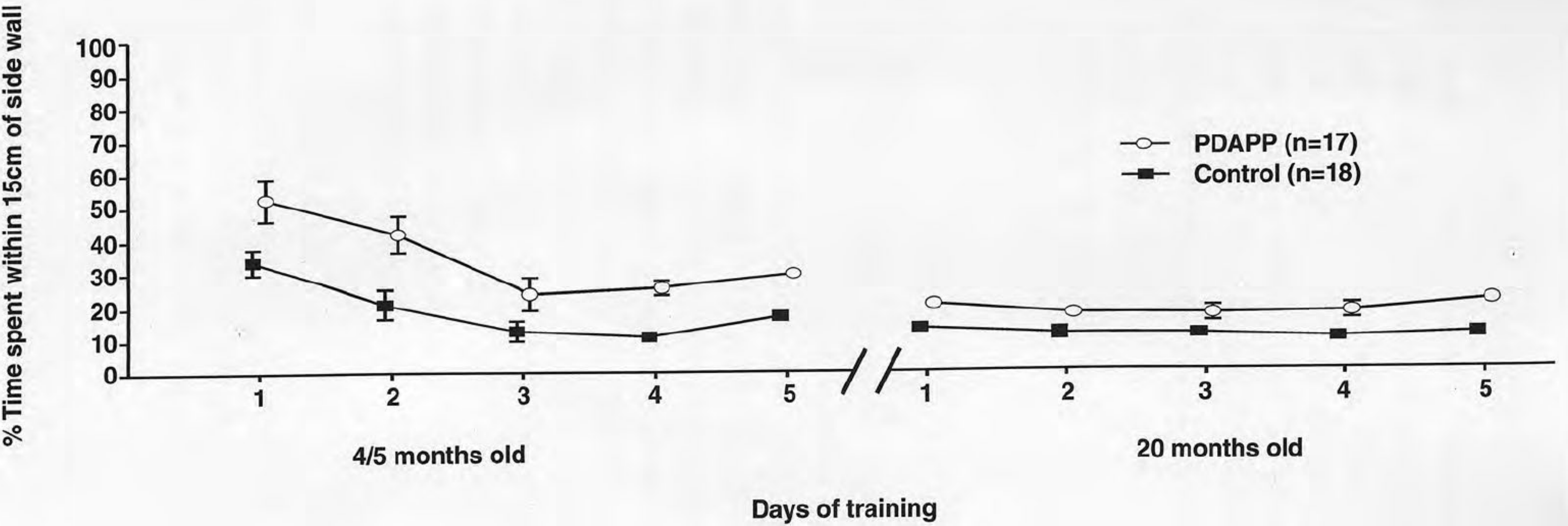


Figure 6.3.4: Thigmotaxis in PDAPP and control animals at both ages tested. PDAPP animals are consistently more thigomtaxic than controls, although by 20 months of age, they are only spending approximately 20% of their time near the side wall.

contribute to the performance observed during the intervening DMP phases. It is interesting to note that the PDAPP group is consistently more thigmotaxic during the first phase of this training. This is the first exposure the animals have had to the pool, and this result could be caused by an increased fear response leading to a different strategy in this task. PDAPP animals show an increased reluctance to swim into the centre of the pool (Controls: day1 - 33.5 ± 3.9% of the trial near the sidewall; day 5 - 16.9 ± 1.3%. PDAPP: day 1 - 52.2 ± 6.3%; day 5 - 29.2 ± 1.5%. Values are means ± standard errors). However, path length does not differ after the first two days, so the PDAPP mice are not taking more circuitous routes round the edge of the pool to the platform.

6.4 DMP Training.

As mentioned previously, the purpose of the variable location days was to establish whether any decrease in latency between trials 1 and 2 was due to the animals learning and remembering the platform's location or whether a lower latency on subsequent trials was for other reasons. Figure 6.4.1 shows the escape latency for control animals only, averaged across all 5 training series. DMP days show the expected decrease in latency between trials 1 and 2; data from variable location days reveals that the decrease in escape latency did not occur until after trial 2. An ANOVA revealed a highly significant effect of trials ($F = 91.88$, df 3/102, $p < 0.0001$) and a conditions (DMP or variable location) by trials interaction ($F = 13.93$, df 3/102, $p < 0.0001$). An uncorrected t-test showed that the escape latency only differed between the conditions on trial 2 ($F = 43.94$, df 1/104, $p < 0.001$).

Having established that normal animals use a spatial learning strategy, the performance of control and PDAPP animals was compared across their entire life-span. This was done by averaging performance in either control or PDAPP groups across the 8 DMP days in each series and then averaging the results across all 5 series

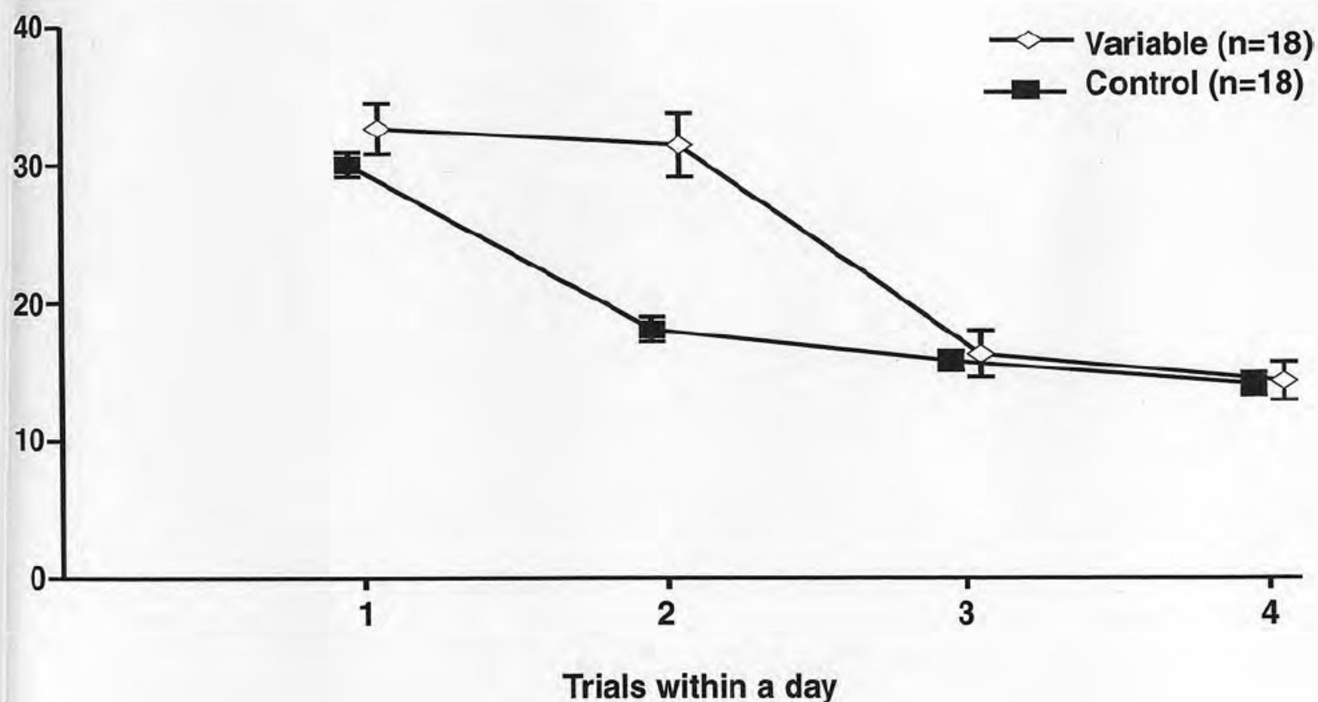


Figure 6.4.1: Time taken to reach the hidden platfrom for control animals only, averaged across all 5 training series. On regular days, controls show a decrease in escape latency between trials 1 and 2. On variable days, when the location of the platform in changed between trials 1 and 2, this decrease does not occur until after trial 2, indicating that control animals are using a spatial strategy to find the platform.

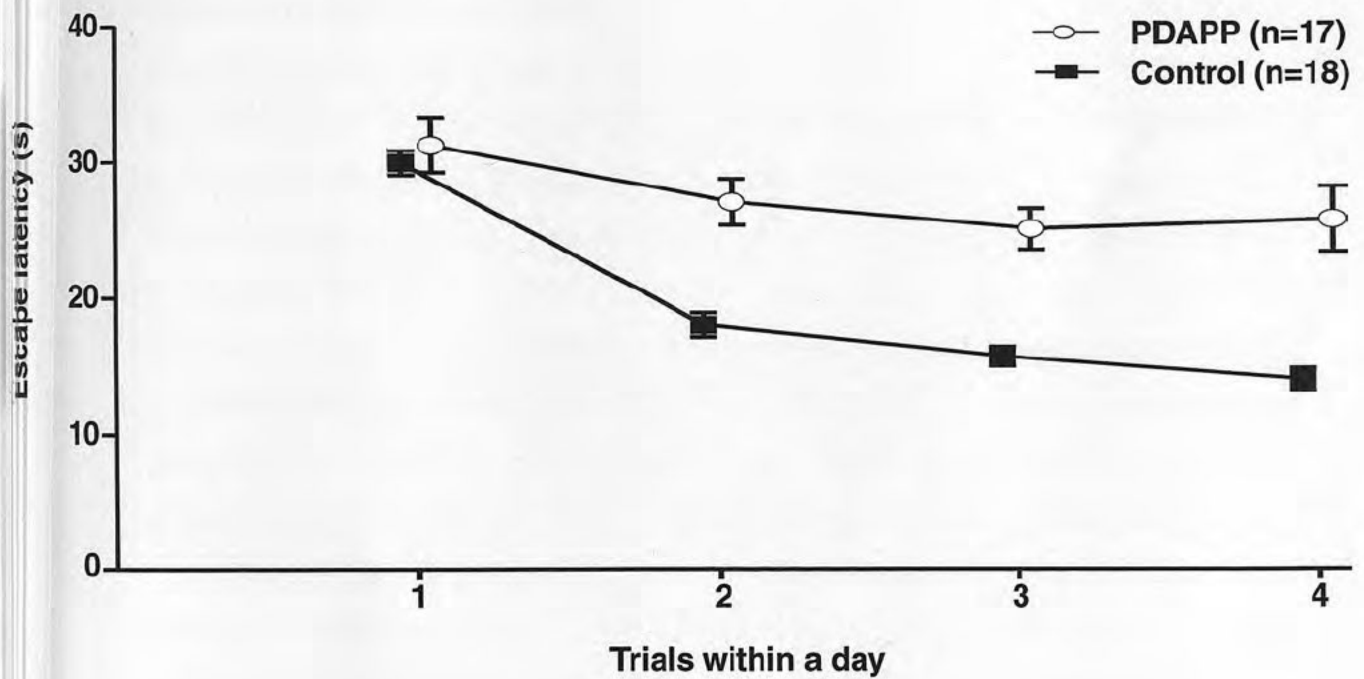


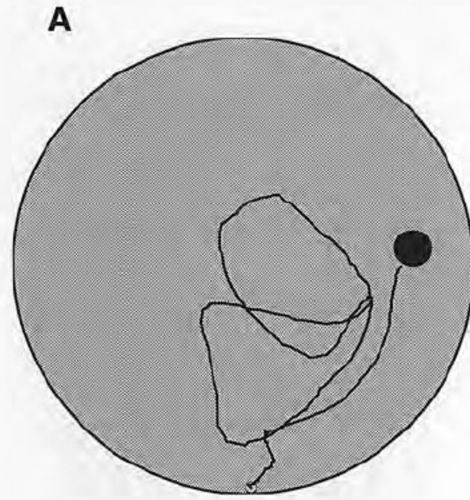
Figure 6.4.2: Time taken to reach the hidden platform for both groups averaged across all 5 training series. The groups are equivalent on trial 1 when the platform is in a new location. Controls show learning between trial 1 and 2, and a slight improvement on trials 3 and 4. PDAPP mice do not learn the location of the platform as their latencies do not decrease.

(from 6 months to 20 months of age). Figure 6.4.2 shows the latency to escape for both groups. PDAPP animals are significantly impaired ($F = 26.3$, df 1/33, $p < 0.0001$). A group by trial interaction was also seen ($F = 19.5$, df 3/99, $p < 0.0001$) indicating that performance of the groups differed across trials within each day. Uncorrected t-tests revealed that both groups were equivalent on the first trial when the location of the platform is new ($F < 1$, $p > 0.4$) but differed significantly on the next three trials (Trial 2: $F = 25.9$, df 1/57, $p < 0.001$; Trial 3: $F = 27.6$, df 1/57; $p < 0.001$; Trial 4: $F = 44.3$, df 1/57, $p < 0.001$). Examples of swim paths are shown in Figures 6.4.3 and 6.4.4.

Analysis of path length produced the same pattern of results (Figure 6.4.5). Overall, a highly significant impairment was seen in the PDAPP group ($F = 19.1$, df 1/33, $p < 0.0005$), a group by trial interaction between both groups ($F = 19.2$, df 3/99, $p < 0.0001$) and uncorrected t-tests revealed that the groups were equivalent on trial 1 but differed thereafter (Trial 1: $F < 1$, $p > 0.5$; Trial 2: $F = 17.8$, df 1/68, $p < 0.001$; Trial 3: $F = 22.1$, df 1/68, $p < 0.001$; Trial 4: $F = 36.1$, df 1/68, $p < 0.001$). No difference in swim speed between the groups was seen throughout training ($F = 1.6$, $p > 0.2$), (Figure 6.4.6). The PDAPP group were, however, consistently more thigmotaxic than the controls (Figure 6.4.7) ($F = 13.6$, df 1/33, $p < 0.0005$). No group by trial interaction was observed ($F = 1.7$, $p > 0.1$) and this was confirmed by uncorrected t-tests which showed that PDAPP animals were more thigmotaxic than controls across all 4 trials (Trial 1: $F = 14.9$, df 1/38, $p < 0.001$; Trial 2: $F = 14.3$, df 1/38, $p < 0.005$; Trial 3 $F = 12.9$, df 1/38, $p < 0.005$; Trial 4: $F = 8.84$, df 1/38, $p < 0.01$).

The data averaged across all 5 training series was then broken down into performance at each age-point tested. Figure 6.4.8A shows the escape latency of the control animals; 6.4.8B that of the PDAPP animals. The control group was remarkably

A



B

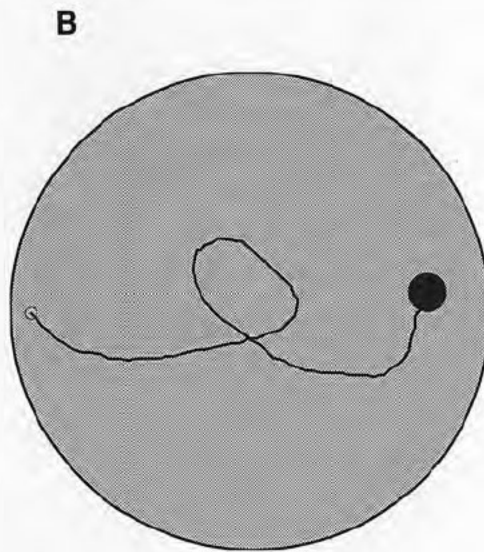


Figure 6.4.3: Typical swim paths for a control animal. The large filled circle represents the watermaze; the small black circle the platform. 6.4.3A shows the path taken on trial 1; 6.4.3B that taken on trial 4. By trial 4, the mouse takes a much shorter route to the platform.

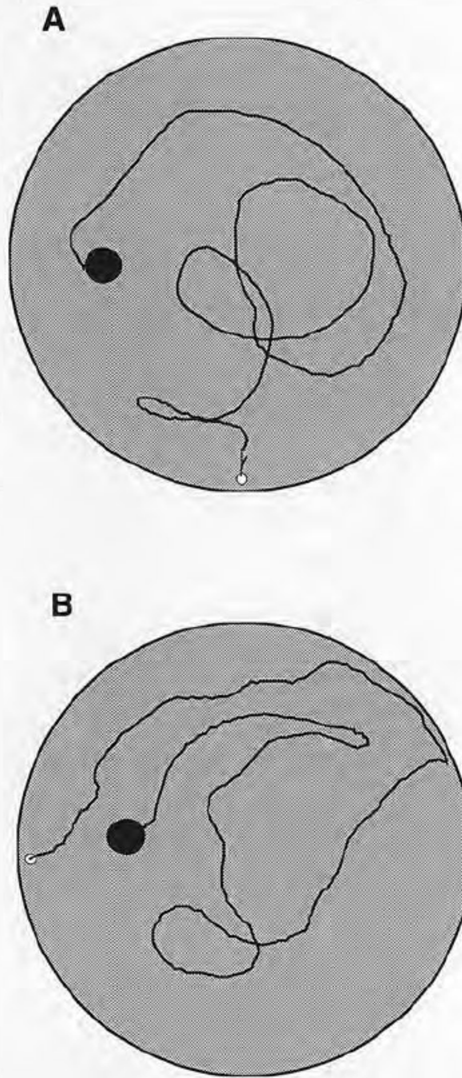


Figure 6.4.4: Typical swim paths for a PDAPP animal. 6.4.4A shows the path taken on trial 1; 6.4.4B that taken on trial 4. In contrast to the control animal, the path taken on trial 4 is still long.

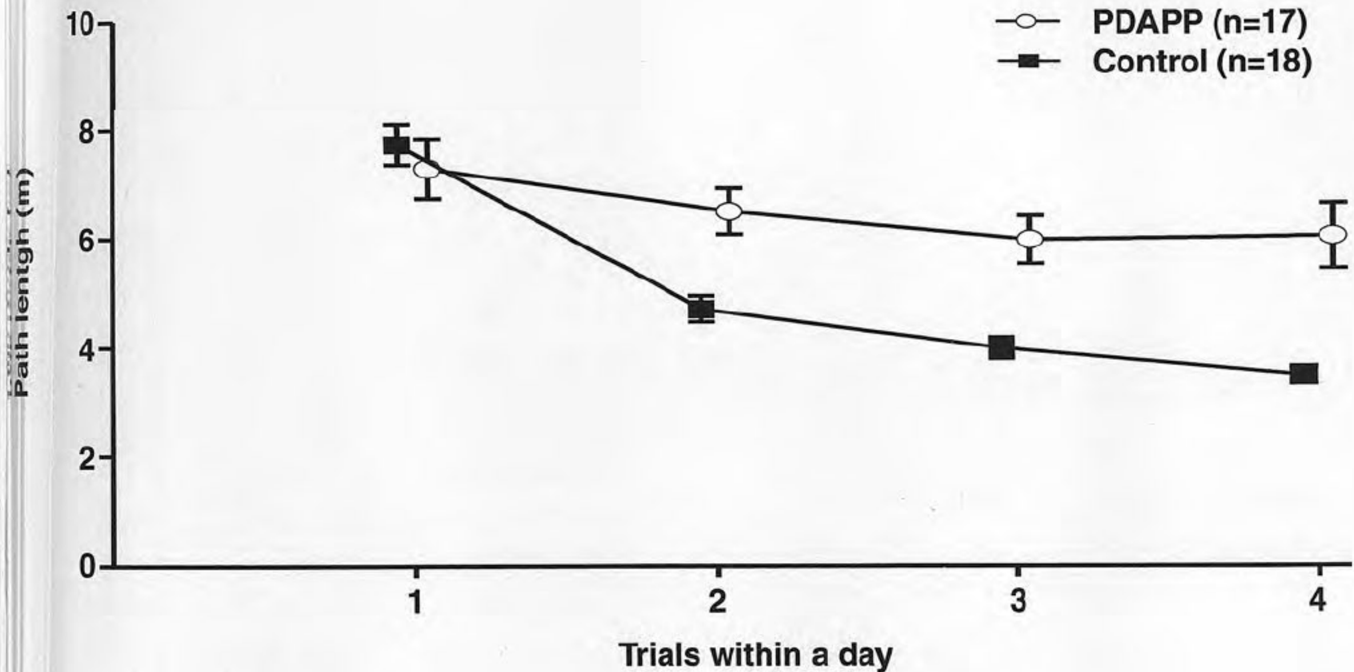


Figure 6.4.5: Distance taken to find the hidden platform for both groups across all 5 training series. The pattern mirrors that of the latency data: groups are equivalent on trial 1 and controls show learning, PDAPP animals do not.

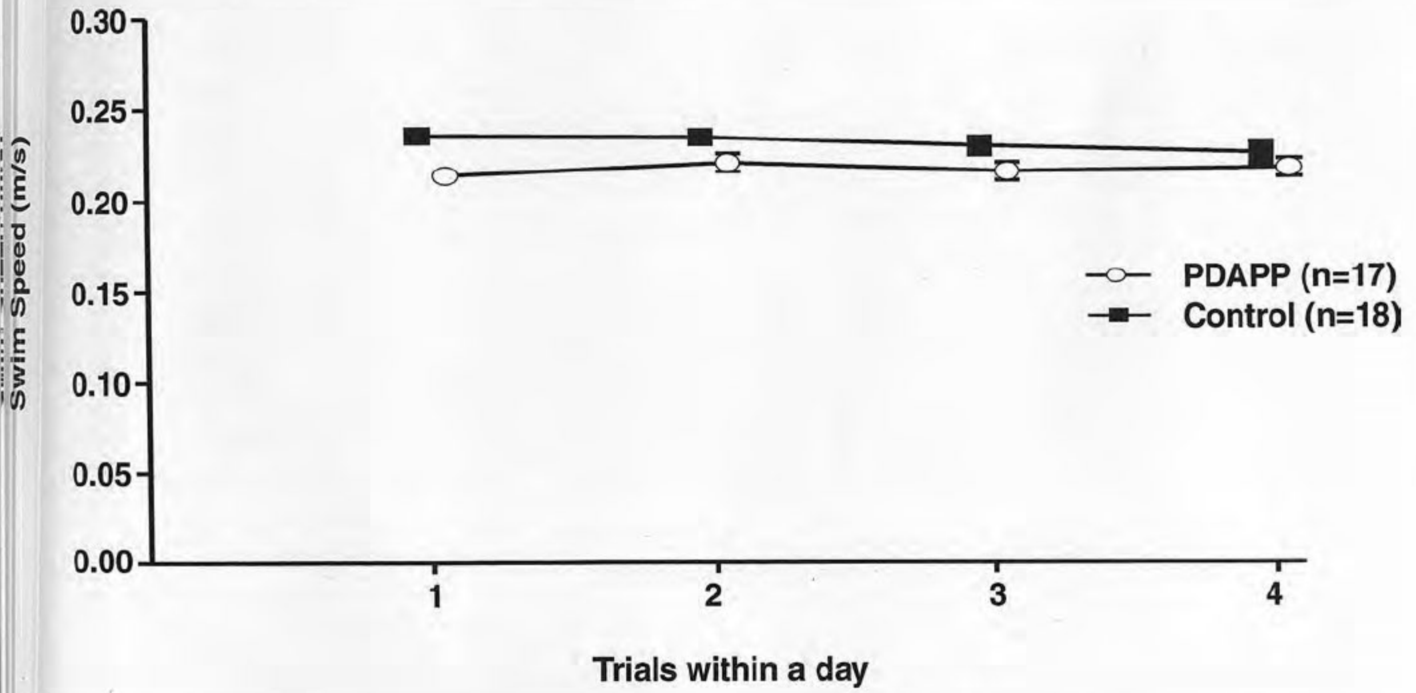


Figure 6.4.6: Swim speed across all 5 training series. There is no difference between the groups.

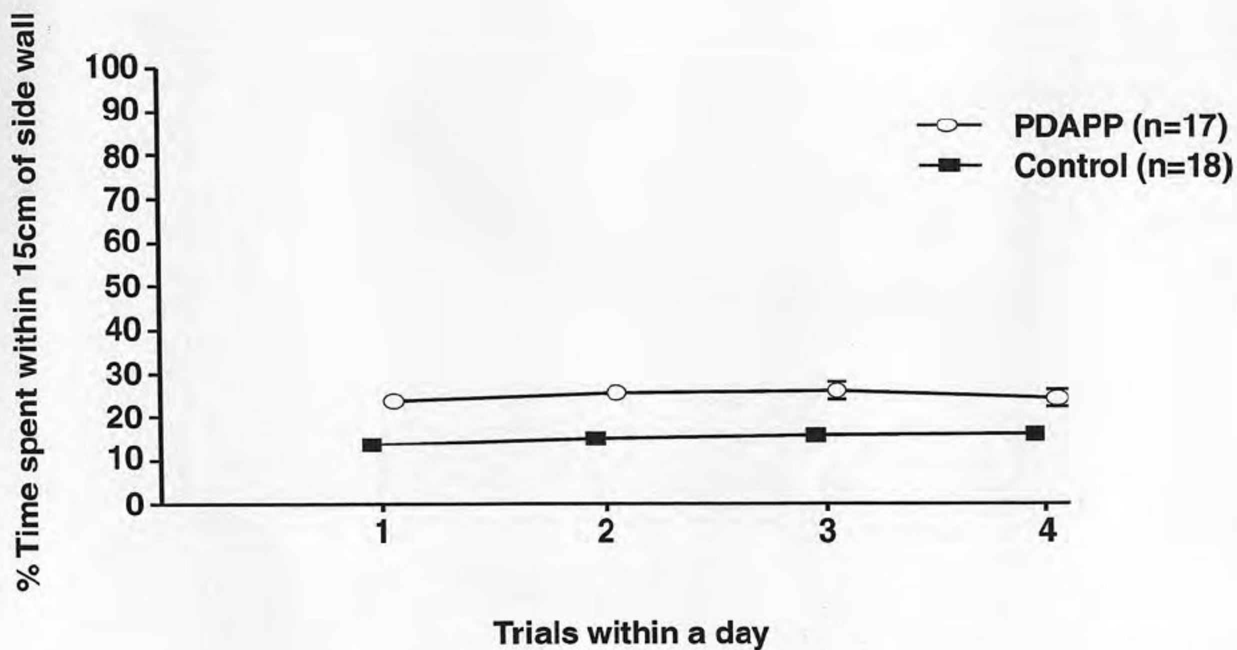


Figure 6.4.7: Thigmotaxis averaged over all 5 training series. PDAPP mice are consistently more thigmotaxic than controls, although they still only spend approximately 25% of their time near the side wall.

Figure 6.4.8A: Performance of control mice broken down by age. Performance is remarkeably consistent across training, with only a slight deterioration in old animals.

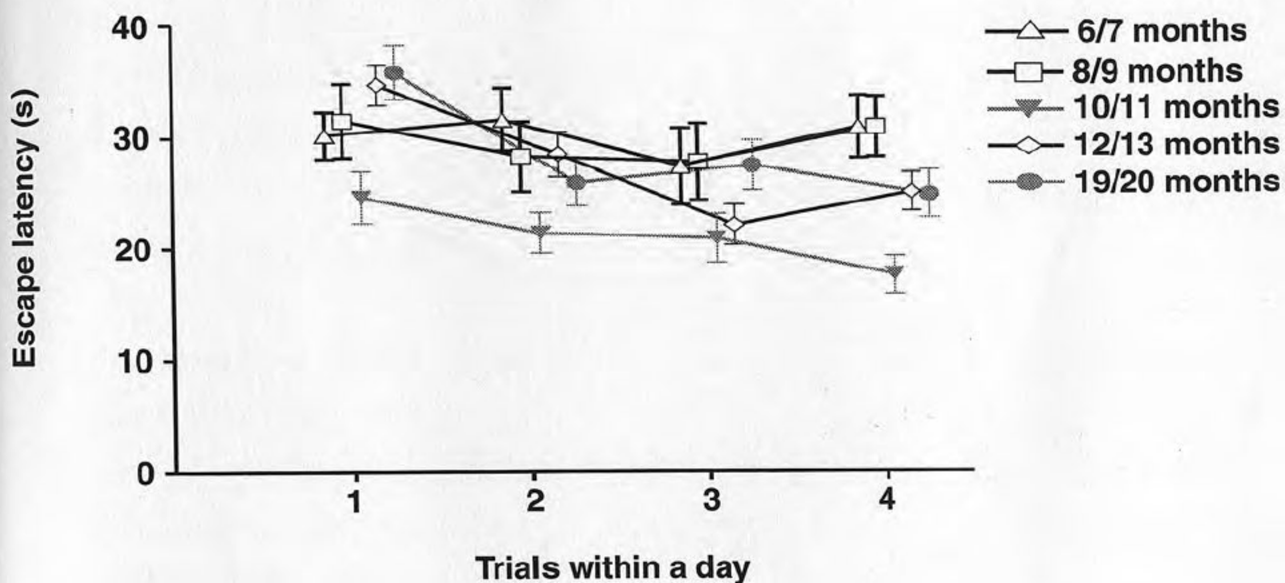


Figure 6.4.8B: Performance of PDAPP mice broken down by age. Performance is variable across training, with no clear indication of learning.

stable in its performance over training. No effect of series was seen ($F = 1.18$, $p > 0.3$) but a large effect of trial was revealed, confirming that learning was occurring across trials ($F = 116.7$, df 3/51, $p < 0.0001$). Performance in the PDAPP group was more variable. A significant effect of series was seen ($F = 6.04$, df 4/64, $p < 0.005$) but inspection of the data shows that this was largely due to unexpectedly lower escape latencies on series 3 (10/11 months of age). All latencies are lower, including trial 1, so this effect does not indicate any learning. An effect of trial is seen, however ($F = 12.5$, df 3/48, $p < 0.0001$). Uncorrected t-tests show that this effect is significant in series 4 and 5 (Series 4: $F = 13.4$, df 3/48, $p < 0.001$; Series 5: $F = 7.39$, df 3/48, $p < 0.001$) indicating, paradoxically, some learning taking place in old PDAPP animals. However, the decrease in latency is seen across all 4 trials in contrast to that in the controls which is primarily between trials 1 and 2 (showing that only they are matching to place).

To summarise, PDAPP animals are impaired in this task, taking longer and more circuitous routes to find the platform than the controls throughout training. They are also consistently more thigmotaxic and their performance, at least measured by escape latency is more variable than controls as they age.

6.5 Histology.

Sections from both control and PDAPP mice were stained. No amyloid deposition or plaques were found from control mice (data not shown). Figures 6.5.1A and 6.5.1B show sections from the hippocampi of PDAPP mice. Amyloid deposition is clearly visible, in the form of brown staining. Plaques can also be seen in figure 6.5.1.A as the more concentrated, darker brown stained areas. Figure 6.5.1.B shows a diffuse plaque at a higher magnification. No dense core is seen, but there are denser areas of staining within it, surrounded by more diffuse staining.

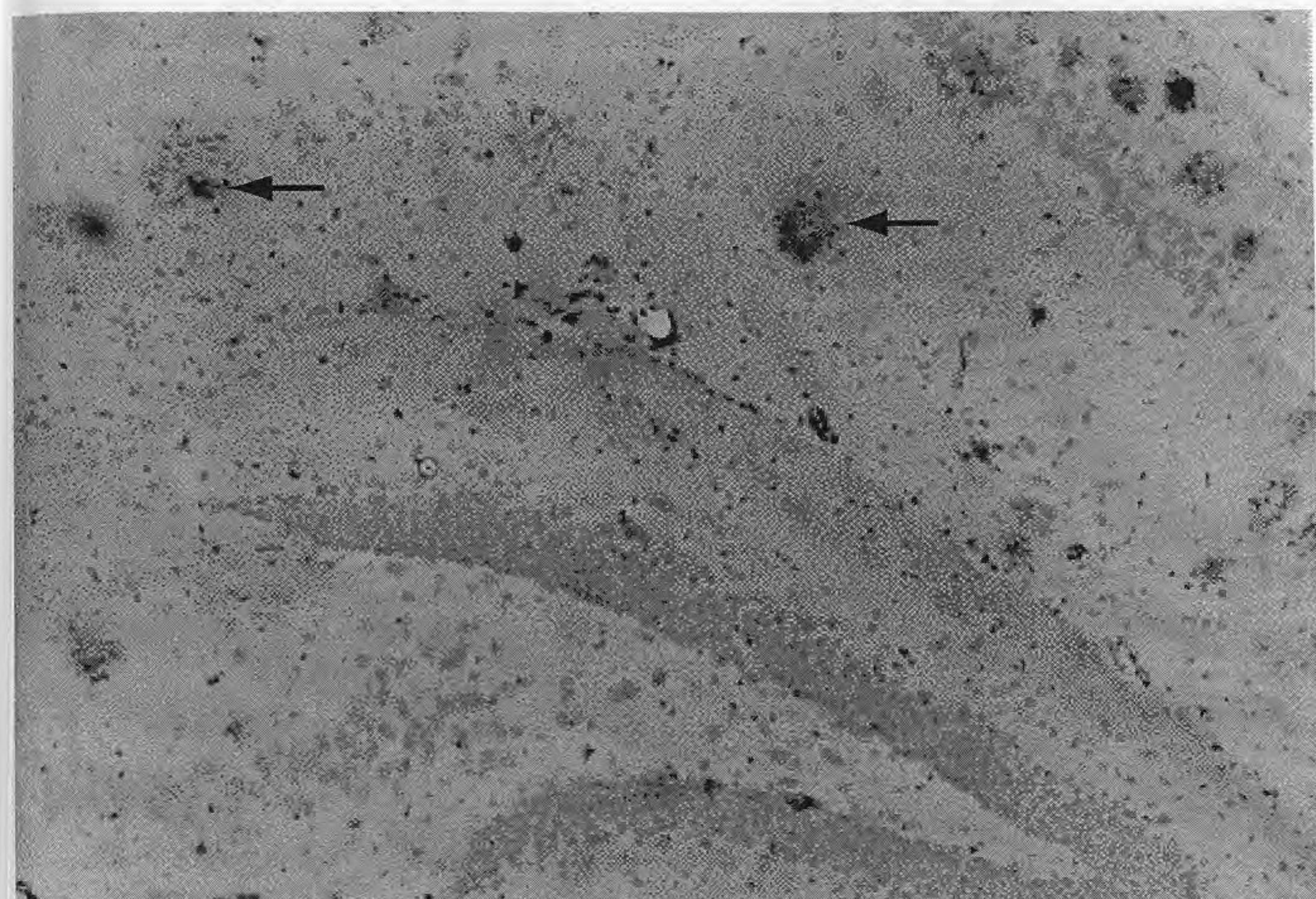


Figure 6.5.1A: Immunostaining of PDAPP mouse brain at age 20 months. Diffuse amyloid deposits can be seen (abve the dentate gyrus) and plaques are also detected (arrows). Antibody 3D6 was used, which detects Aβ 1-5. The section was counterstained with haemotoxylin. Magnification x 100.

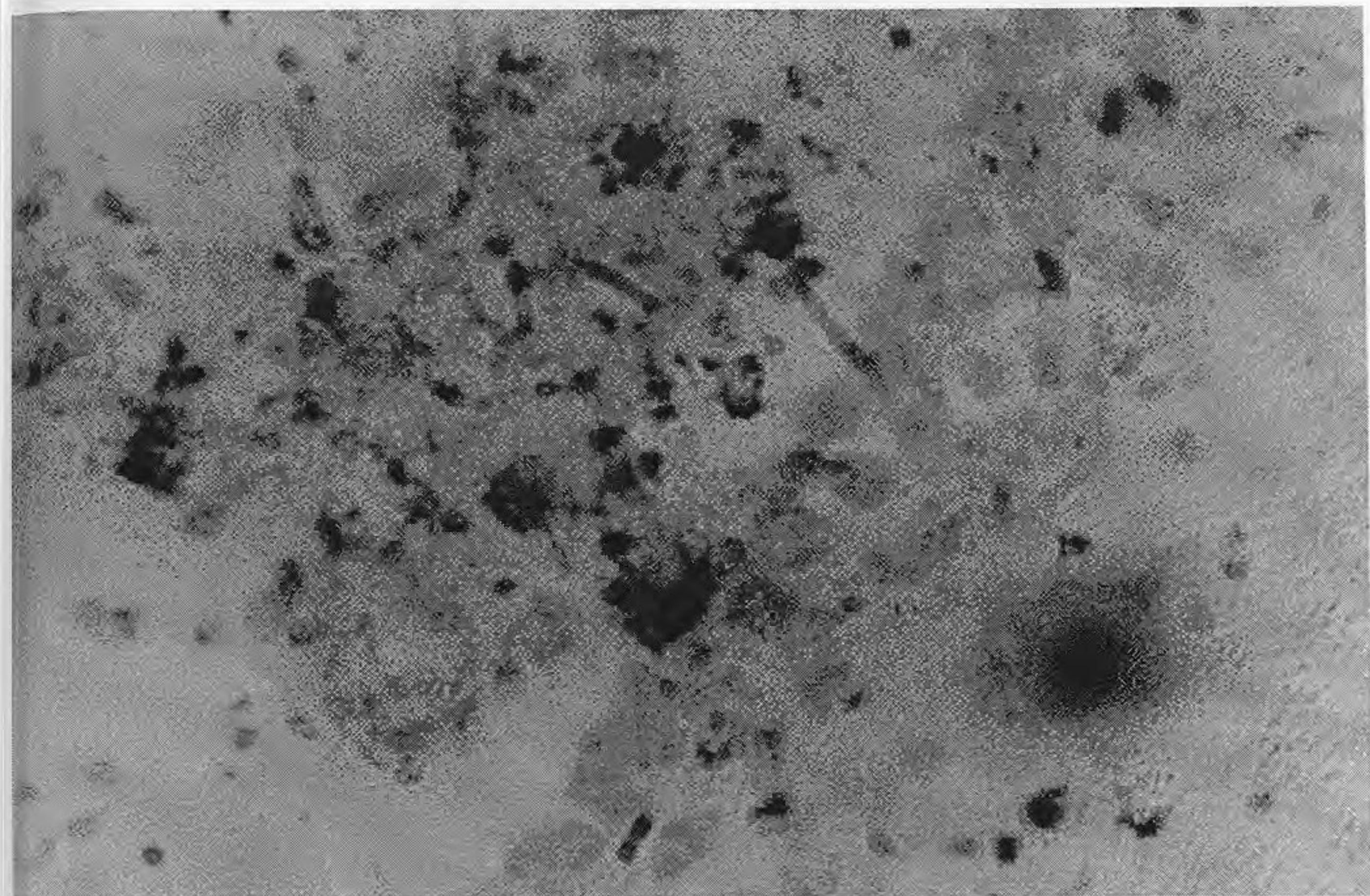


Figure 6.5.1B: Immunostaining of PDAPP mouse brain at age 20 months. A diffuse plaque in the hippocampus. Antibody 3D6 was used, which detects Aβ 1-5. The section was counterstained with haemotoxylin. Magnification x 400.

7. Conclusions.

It had been planned to use the DMP protocol to look for age- or plaque-related changes in performance. However, the only firm conclusions which can be drawn from these data are that (a) overexpression of human V717F APP is sufficient to cause a learning deficit ; (b) the presence of neuritic plaques is not a necessary condition and (c) control mice can match to place. PDAPP animals are impaired from the earliest age tested, so no baseline performance was established with which to compare any loss of performance with as the animals aged. Had plaque formation been critical, some learning may have been seen in the first series (6/7 months of age) and then performance declined as more plaques formed in the hippocampus and neocortex.

8. Discussion.

No age-related impairment was seen in this study. Justice and Motter (1997) found impairments in spatial learning in PDAPP mice as early as 2 months. One possible explanation is that the overexpression of mutant hAPP itself is sufficient to cause a learning deficit. The levels of APP in this model are ≥ 10-fold higher than either endogenous mouse brain or those found in AD brain (Games, Adams et al. 1995). The choice of promoter and the inclusion of introns 6-8 in the construct allow the alternatively spliced isoforms APP_{751} and APP_{770} to be expressed in brain as well as the neuronal-specific APP_{695}. It is possible that such high levels of APP itself may disrupt normal function in the brain and produce a learning deficit, before any major Aβ aggregation.

To address some of these issues, control experiments could be performed with the following mice. A mouse which overexpresses normal murine APP could be generated to ascertain whether mere overexpression causes any effect. Overexpression of normal human APP in a mouse may also be useful in answering this question. It may also be possible to generate a mouse carrying human APP but no mouse APP. However, this may complicate interpretation further if human APP does not replace the function of mouse APP. Murine APP knockouts are viable, but show impairments in learning and LTP (Dawson, Seabrook et al. 1997).

The task may not be sensitive enough to detect any age-related deficit that is present. Even from the youngest age tested in the DMP task (6/7 months), PDAPP animals were unable to perform well. They never learned to adopt a spatial search strategy. It is interesting to note the thigmotaxis results. In control animals (Figure 6.4.6) the amount of time spent near the side walls does not change during later trials in the day when they are taking shorter, more direct paths to the platform. The implication is that the absolute duration for which a control animal stays near the side wall decreases in

proportion to how well that animal knows where the platform is hidden. This suggests that thigmotaxis is not just a sensorimotor side-effect (which it can be in some cases e.g. drug treatment) but may also be a behavioural disposition which is influenced by the animal's knowledge of the training situation. The PDAPP group have failed to acquire a good strategy to navigate to the platform, and so stay near the side wall. The converse is also possible: that the task requirements are not demanding enough for the PDAPP animals. This sounds counter-intuitive, but if these animals have a lack of motivation to escape, then they would circle the pool instead of heading to the platform.

To overcome these problems, more daily training trials could be given in the DMP task to allow the PDAPP animals to learn as the data show they cannot learn the task in 4 trials. They could even be trained until they reached a certain criterion. This would provide an additional measure of performance: the number of trials required to reach this criterion. The PDAPP group need not show learning to an equivalent standard as the controls (although this would be ideal, but highly unlikely) so long as some evidence of learning was present in young animals. Training the animals at a younger age (2-3 months old) may also help.

Consistent with other reports of transgenic models overexpressing mutant human APP (Hsiao, Chapman et al. 1996), the PDAPP mouse is impaired in spatial learning and memory, at least in a DMP task. Much more behavioural characterisation needs to be performed to understand the nature of this deficit, how specific it is and when exactly it appears. Other tests need to be administered to discover whether there are any changes in fear response, sensory effects, motivation or other effects that may affect performance.

Although this model shares many features with AD, it is at best a partial model of the disease. No tangle formation is seen and there is not the same pattern of cell loss present as in the disease (Games, Adams et al. 1995; Irizarry, Soriano et al. 1997). However, the age dependent appearance of neuritic plaques, their composition and the inflammatory response surrounding them are all typical of AD. The model will be useful in understanding plaque formation, the metabolism of APP, and certainly contributes to the debate whether Aβ deposition is central to the disease process or is a 'side effect' of the disease. Further characterisation of the PDAPP mouse is essential if compounds which putatively decrease or block Aβ production are to be tested. All the models have their own strengths and weaknesses but correct interpretation of the data already presented from them and those to come will, further our understanding of at least one area of the disease process.

9. References.

Abraham, C. R., D. J. Selkoe, et al. (1988). "Immunochemical identification of the serine protease inhibitor, α_1 - antichymotrypsin in the brain amyloid deposits of Alzheimer's disease." Cell **52**: 487-501.

Agid, Y., M. Ruberg, et al. (1987). Anatomoclinical and biochemical concepts of subcortical dementia. Cognitive Neurochemistry. S. M. Stahl, S. D. Iversen and E. C. Goodman, Oxford University Press: 248-271.

Arendt, T., M. Holzer, et al. (1995). "Paired helical filament-like phosphorylation of tau, deposition of beta/A4-amyloid and memory impairment in rat induced by chronic inhibition of phosphatase 1 and 2A." Neuroscience **69**: 691-698.

Bartus, R. T., R. L. Dean, et al. (1982). "The cholinergic hypothesis of geriatric memory dysfunction." Science **217**: 408-417.

Battaini, F., R. Del Vasco, et al. (1990). "Regulation of phorbol ester binding and protein kinase C activity in aged rat brain." Neurobiology of Aging **11**: 563-566.

Beal, M. F., L. C. Walker, et al. (1991). "Neurotransmitters in neocortex of aged rhesus monkeys." Neurobiology of Aging **12**: 407-412.

Benzi, G. and A. Moretti (1995). "Are reactive oxygen species involved in Alzheimer's disease?" Neurobiology of Aging **16**: 611-674.

Berezovska, O., M. Q. Xia, et al. (1997). "Developmental regulation of presenilin mRNA expression parallels Notch expression." Journal of Neuropathology and Experimental Neurology **56**: 40-44.

Borchelt, D. R., G. Thinakaran, et al. (1996). "Familial Alzheimer's disease-linked presenilin 1 variants elevate Aβ 1-42/1-40 ratio in vitro and in vivo." Neuron **17**: 1005-1013.

Braak, H., E Braak, et al. (1996). "Pattern of brain destruction in Parkinson's and Alzheimer's diseases." Journal of Neural Transmission **103**: 455-490.

Buxbaum, J. D., J. L. Christensen, et al. (1993). "Expression of APP in brains of transgenic mice containing the entire human APP gene." Biophysical and Biochemical Research Communications **197**: 639-645.

Buxbaum, J. D., A. A. Ruefly, et al. (1994). "Calcium regulates processing of the Alzheimer amyloid protein precursor in a protein kinase C-independent manner." Proceedings of the National Academy of the Sciences USA **91**: 9195-9198.

Cain, D. P., D. Saucier, et al. (1996). "Detailed behavioral analysis of water maze acquisition under APV or CNQX: contribution of sensorimotor disturbances to drug-induced acquisition deficits." Behavioral Neuroscience **110**(1): 86-102.

Chapman, P. F., G. White, et al. (1998). "APP Transgenic mice - from behaviour to physiology (and back)." European Journal of Neuroscience Supplement **10**: 135.01.

Cole, G., K. R. Dobkins, et al. (1988). "Decreased levels of protein kinase C in Alzheimer brain." Brain Research **453**: 165-174.

Cook, D. B., J. C. Sung, et al. (1996). "Expression and analysis of presenilin1 in a human neuronal system: Localisation in cell bodies and dendrites." Proceedings of the National Academy of the Sciences USA **93**: 9223-9228.

Corder, E. H., A. M. Saunders, et al. (1994). "Protective effect of apolipoprotein E type 2 allele for late onset Alzheimer disease." Nature Genetics **7**: 180-183.

Corder, E. H., A. M. Saunders, et al. (1993). "Gene dose of apolipoprotein E type 4 allele and the risk of Alzheimer's disease in late onset families." Science **261**: 921-923.

Cork, L. C., C. Masters, et al. (1990). "Development of senile plaques. Relationships of neuronal abnormalities and amyloid deposits." American Journal of Pathology **137**: 1383-1392.

Cowburn, R. F., C. O'Neill, et al. (1992). "Adenylyl cyclase activity in post-mortem human brain: evidence of altered G protein mediation in Alzheimer's disease." Journal of Neurochemistry **58**: 1409-1419.

Coyle, J. T., D. L. Price, et al. (1983). "Alzheimer's disease: a disorder of cortical cholinergic innervation." Science **219**: 1184-1190.

Dahl, D., A. Bignami, et al. (1980). "Immunohistochemical characterisation of neurofibrillary tangles induced by mitotic spindle inhibitors." Acta Neuropathologica **51**: 165-168.

Dawson, G. R., G. R. Seabrook, et al. (1997). "Age-related cognitive deficits, impaired long-term potentiation and reduction in synaptic marker density in mice lacking the β-amyloid precursor protein." Society for Neuroscience Abstracts **23**: 442.10.

Duff, K., C. Eckman, et al. (1996). "Increased amyloid-β42(43) in brains of mice expressing mutant presenilin 1." Nature **383**: 710-713.

Dyrks, T., U. Monning, et al. (1994). "Amyloid precursor protein secretion and βA4 amyloid generation are not mutually exclusive." FEBS Letters **349**: 210-214.

Efthimiopoulos, S., K. M. Felsenstein, et al. (1994). "Study of the phorbol ester effect on Alzheimer amyloid precursor processing: sequence requirements and involvement of a cholera toxin sensitive protein." Journal of Neuroscience Research **38**: 81-90.

Efthimiopoulos, S., S. Punj, et al. (1996). "Intracellular cyclic AMP inhibits constitutive and phorbol ester-stimulated secretory cleavage of amyloid precursor protein." Journal of Neurochemistry **67**: 872-875.

Elder, G. A., N. Tezapsidis, et al. (1996). "Identification and neuron specific expression of the S182/presenilin 1 protein in human and rodent brains." Journal of Neuroscience Research **45**: 308-320.

Ferrari-DiLeo, G. and D. D. Flynn (1993). "Diminished muscarinic receptor-stimulated $[^3H]$-PIP_2 hydrolysis in Alzheimer's disease." Life Sciences **53**: 439-444.

Fowler, C. J., R. F. Cowburn, et al. (1997). "Alzheimer's, ageing and amyloid: and absurd allegory?" Gerontology **43**: 132-142.

Fowler, C. J., A. Garlind, et al. (1996). "Receptor-effector coupling dysfunctions in Alzheimer's disease." Annals of the New York Academy of Sciences **786**: 294-304.

Games, D., D. Adams, et al. (1995). "Alzheimer-type neuropathology in transgenic mice overexpressing V717F β-amyloid precursor protein." Nature **373**: 523-526.

Garlind, A., R. F. Cowburn, et al. (1995). "Diminished $[^3H]$inositol(1,4,5)P_3 but not $[^3H]$inositol(1,3,4,5)P_4 binding in Alzheimer's disease brain." Brain Research **681**: 160-166.

Gegelashvili, G., E. Bock, et al. (1996). "Two types of amyloid precursor protein (APP) mRNA in rat glioma cell lines: upregulation via a cyclic AMP-dependent pathway." Molecular Brain Research **37**: 151-156.

Goldman-Rakic, P. S. and R. M. Brown (1981). "Regional changes of monoamines in cerebral cortex and subcortical structures of aging rhesus monkeys." Neuroscience **6**: 177-187.

Grunde-Iqbal, I., K. Iqbal, et al. (1986). "Abnormal phosphorylation of the microtubule-associated protein τ (tau) in Alzheimer cytoskeletal pathology." Proceedings of the National Academy of the Sciences USA **83**: 4913-4917.

Guo, Q., K. Furukawa, et al. (1996). "Alzheimer's PS-1 mutation perturbs calcium homoeostasis and sensitises PC12 cells to death induced by amyloid β-peptide." NeuroReport **8**: 379-383.

Guo, S., B. L. Sopher, et al. (1997). "Alzheimer's presenilin mutations sensitises neural cells to apoptosis induced by trophic factor withdrawal and amyloid β-peptide: involvement of calcium and oxyreadicals." The Journal of Neuroscience **17**: 4212-4222.

Hardy, J. (1997). "Amyloid, the presenilins and Alzheimer's disease." Trends in the Neurosciences **20**: 154-159.

Hardy, J. (1994). "Alzheimer's disease: clinical molecular genetics." Clinics in Geriatric Medicine **10**: 239-247.

Horsburgh, K. and T. Saitoh (1994). Altered signal transduction in Alzheimer's disease. Alzheimer's disease. R. D. Terry, R. Katzman and K. L. Bick. New York, Raven Press: 387-404.

Hung, A. Y. and D. J. Selkoe (1994). "Selective ectodomain phosphorylation and regulated cleavage of beta-amyloid precursor protein." EMBO Journal **13**(3): 534-42.

Hsiao, K., P. Chapman, et al. (1996). "Correlative memory deficits, Aβ elevation, and amyloid plaques in transgenic mice." Science **274**: 99-102.

Irizarry, M. C., F. Soriano, et al. (1997). "Aβ deposition is associated with neuropil changes, but not with overt neuronal loss in the human amyloid precursor protein V717F (PDAPP) transgenic mouse." The Journal of Neuroscience **17**: 7053-7059.

Jarrett, J. T., E. P. Berger, et al. (1993). "The carboxy terminus of the beta amyloid protein is critical for the seeding of amyloid formation: implications for the pathogenesis of Alzheimer's disease." Biochemistry **32**: 4693-4697.

Johnson-Wood, K., M. Lee, et al. (1997). "Amyloid precursor protein processing and $A\beta_{42}$ deposition in a transgenic mouse model of Alzheimer's Disease." Proceedings of the National Academy of the Sciences USA **94**: 1550-1555.

Jolly-Tornetta, C., Z.-y. Gao, et al. (1998). "Regulation of amyloid precursor protein secretion by glutamate receptors in human Ntera 2 neurons (NT2N)." The Journal of Biological Chemistry **273**: 14015-14021.

Justice, A. and R. Motter (1997). "Behavioral characterization of PDAPP transgenic Alzheimer mice." Society for Neuroscience Abstracts **23**: 636.6.

Kovacks, D. M., H. J. Fausett, et al. (1996). "Alzheimer-associated presenilins 1 and 2: Neuronal expression in brain and localisation to intracellular membranes in mammalian cells." Nature Medicine **2**: 224-229.

Lah, J. J., C. J. Heilman, et al. (1997). "Light and electron microscopic localisation of presenilin-1 in primate brain." The Journal of Neuroscience **17**: 1971-1980.

Lee, R. K. K., R. J. Wurtman, et al. (1995). "Amyloid precursor protein is stimulated by metabotropic glutamate receptors." Proceedings of the National Academy of Sciences of the United States of America **92**: 8083-8087.

Lee, M. K., H. H. Slunt, et al. (1996). "Expression of presenilin 1 and 2 (PS1 and PS2) in human and murine tissues." The Journal of Neuroscience **16**: 7513-7525.

Lehmann, S., R. Chiesa, et al. (1997). "Evidence for a six-transmembrane domain structure of presenilin 1." The Journal of Biological Chemistry **272**: 12047-12051.

Levitan, D., T. G. Doyle, et al (1996). "Assessment of normal and mutant human presenilin function in *Caenorhabditis elegans*." Proceedings of the National Academy of the Sciences USA **93**: 14940-14944.

Levitan, D. and I. Greenwald (1995). "Facilitation of lin-12 mediated signalling by *sel*-12, a *Caenorhabditis elegans* S182 Alzheimer's disease gene." Nature **377**: 351-354.

Levy-Lahad, E., W. Wasco, et al. (1995). "Candidate gene for the chromosome 1 familial Alzheimer's disease locus." Science **269**: 973-977.

Levy-Lahad, E., E. M. Wijsman, et al. (1995). "A familial Alzheimer's disease locus on chromosome I." Science **269**: 970-973.

L'Hernault, S. W. and P. M. Arduengo (1992). "Mutation of a putative sperm membrane protein in *Caenorhabditis elegans* prevents sperm differentiation but not associated meiotic divisions." J. Cell. Biol. **119**: 55-68.

Li, X. and I. Greenwald (1996). "Membrane topology of the *C. elegans* SEL-12 presenilin." Neuron **17**: 1015-1021.

Mahley, R. W. (1998). "Apolipoprotein E: cholesterol transport with expanding role in cell biology." Science **240**: 622-630.

Martin, L. J., S. S. Sisodia, et al. (1991). "Amyloid precursor protein in aged nonhuman primates." Proceedings of the National Academy of the Sciences USA **88**: 1461-1465.

Masliah, E., M. Mallory, et al. (1993). "Phorbol-ester induced neuritic alterations in the rat neocortex. Structural and immunocytochemical studies." Molecular and Chemical Neuropathology **20**: 125-145.

Matthies, H. G. J., H. C. Palfrey, et al. (1987). "Down regulation of protein kinase C in neuronal cells: effects on neurotransmitter release." The Journal of Neuroscience **7**: 1198-1208.

Mattson, M. P. (1997a). "Cellular actions of β-amyloid precursor protein and its soluble and fibrillogenic derivatives." Physiological Reviews **77**: 1081-1132.

Mattson, M. P. (1997b). "Central role of oxyradicals in the mechanism of amyloid β-peptide cytotoxicity." Alzheimer's Disease Review **2**: 1-14.

Mattson, M. P. and Q. Guo (1997). "Cell and molecular neurobiology of presenilins: a role for the endoplasmic reticulum in the pathogenesis of Alzheimer's Disease?" Journal of Neuroscience Research **50**: 505-513.

Mattson, M. (1991). "Evidence for the involvement of protein kinase C in neurodegenerative changes in cultured human cortical neurons." Experimental Neurology **112**: 95-103.

Mills, J., D. L. Charest, et al. (1997). "Regulation of amyloid precursor protein catabolism involves the mitogen-activated protein kinase signal transduction pathway." The Journal of Neuroscience **17**: 9415-9422.

Morris, R. G. M. (1984). "Developments of a water-maze procedure for studying spatial learning in the rat." Journal of Neuroscience Methods **11**: 47-60.

Morris, R. G. M., P. Garrud, et al. (1982). "Place navigation impaired in rats with hippocampal lesions." Nature **297**: 681-683.

Namba, Y., M. Tomonaga, et al. (1991). "Apolipoprotein E immunoreactivity in cerebreal amyloid deposits and neurofibrillary tangles in Alzheimer's disease and kuru plaque amyloid in Creuztfeldt-Jacob disease." Brain Research **541**: 163-166.

Nukina, N. and Y. Ihara (1986). "One of the antigenic determinants of paired helical filaments is related to tau protein." Journal of Biochemistry **99**: 1541-1544.

Ohyagi, Y., K. Takahashi, et al. (1990). "Developmental and differential expression of beta amyloid protein precursor mRNAs." Biophysical and Biochemical Research Communications **167**: 54-60.

Pacheco, M. A. and R. S. Jope (1996). "Phosphinositide signalling in the human brain." Progress in Neurobiology **50**: 255-73.

Page, K., R. Hollister, et al. (1996). "In situ hybridisation of presenilin 1 mRNA in Alzheimer's disease and lesioned rat brain." Proceedings of the National Academy of the Sciences USA **93**: 14020-14024.

Probst, A., B. H. Anderson, et al. (1989). "Senile plaque neurites fail to demonstrate anti-paired helical filament and anti-microtubule-associated protein-tau immunoreactive proteins in the absence of neurofibrillary tangles in the neocortex." Acta Neuropathologica **77**: 430-436.

Quon, D., Y. Wang, et al. (1991). "Formation of beta-amyloid protein deposits in brains of transgenic mice." Nature **352**: 239-241.

Rogers, J., N. R. Cooper, et al. (1992). "Complement activation by β-amyloid in Alzheimer disease." Proceedings of the National Academy of the Sciences USA **89**: 10016-10020.

Ross, B. M., M. McLaughlin, et al. (1993). "Alterations in the activity of adenylate cyclase and high affinity GTPase in Alzheimer's disease." Brain Research **622**: 35-42.

Sanan, D. A., K. H. Weisgraber, et al. (1994). "Apolipoprotein E associates with β amyloid peptide of Alzheimer's disease to form novel monofibrils. Isoform ApoE4 associates more efficiently than ApoE3." Journal of Clinical Investigation **94**: 860-869.

Saunders, A. M., W. J. Strittmatter, et al. (1993). "Association of apolipoprotein E alleleε4 with late-onset familial and sporadic Alzheimer's disease." Neurology **43**: 1467-1472.

Schenk, D., E. Masliah, et al. (1997). "The PDAPP transgenic mouse as an animal model for Aβ-induced amyloidosis and neuropathology." Alzheimer's Disease Review **2**: 20-27.

Scheuner, D., C. Eckman, et al. (1996). "Secreted amyloid β-protein similar to that in the senile plaques of Alzheimer's disease is increased *in vivo* by the presenilin 1 and 2 and *APP* mutations linked to familial Alzheimer's disease." Nature Medicine **2**: 864-870.

Schmechel, D. E., A. M. Saunders, et al. (1993). "Increased amyloid β–peptide deposition in cerebral cortex as a consequence of apolipoprotein E genotype in late-onset Alzheimer disease." Proceedings of the National Academy of the Sciences USA **90**: 9649-9653.

Selkoe, D. J. (1994). "Normal and abnormal biology of the β-amyloid precursor protein." Annual Review of Neuroscience **17**: 489-517.

Selkoe, D. J., D. S. Bell, et al. (1987). "Conservation of brain amyloid proteins in aged mammals and humans with Alzheimer's disease." Science **235**: 873-877.

Sherrington, R., E. I. Rogaev, et al. (1995). "Cloning of a gene bearing mis-sense mutations in early-onset familial Alzheimer's disease." Nature **375**: 754-760.

Strittmatter, W. J., A. M. Saunders, et al. (1993a). "Apolipoprotein E: high avidity binding to β-amyloid and increased frequency of type 4 allele in late-onset familial Alzheimer disease." Proceedings of the National Academy of the Sciences USA **90**: 1977-1981.

Strittmatter, W. J., K. H. Weisgraber, et al. (1993b). "Binding of human apolipoprotein E to synthetic amyloid β peptide: isoform-specific effects and implications for late-onset Alzheimer disease." Proceedings of the National Academy of the Sciences USA **90**: 8098-8102.

Suzuki, T., S. Nishiyama, et al. (1996). "Regional and cellular presenilin 1 gene expression in human and rat tissues." Biophysical and Biochemical Research Communications **219**: 708-713.

Shimohama, S., M. Narita, et al. (1993). "Assessment of protein kinase C isozymes by two-site enzyme immunoassay in human brains and changes in Alzheimer's disease." Neurology **43**: 1407-1413.

Slack, B. E., R. M. Nitsch, et al. (1993). "Regulation by phorbol esters of amyloid precursor protein release from Swiss 3T3 fibroblasts overexpressing protein kinase Cα." The Journal of Biological Chemistry **268**: 21097-21101.

Snow, A. D., H. Mar, et al. (1988). "The presence of heparan sulphate proteoglycans in the neuritic plaques and congophilic angiopathy in Alzheimer's disease." American Journal of Pathology **133**: 456-463.

Stabel, S., A. Rodriguez-Pena, et al. (1987). "Quantitation of protein kinase C by immunoblot expression in different cell lines and response to phorbol esters." Journal of Cellular Physiology **130**: 111-116.

Stokes, C. E. and J. N. Hawthorn (1987). "Reduced phosphoinositide concentration in anterior temporal cortex of Alzheimer-diseased brains." Journal of Neurochemistry **48**: 1018-1021.

Struble, R. G., D. L. J. Price, et al. (1985). "Senile plaques in cortex of aged normal monkeys." Brain Research **361**: 267-275.

Sturchler-Pierrat, C., D. Abramowski, et al. (1997). "Two amyloid precursor protein transgenic mouse models with Alzheimer disease-like pathology." Proceedings of the National Academy of the Sciences USA **94**: 13287-13292.

Suzuki, N., T. T. Cheung, et al. (1994). "An increased percentage of long amyloid β protein secreted by familial amyloid β protein precursor (βAPP_{717}) mutants." Science **264**: 1336-1340.

Suzuki, T., K. Ando, et al. (1997). "Phosporylation of Alzheimer β-amyloid precursor-like protein." Biochemistry **36**: 4643-4649.

Tanzi, R. E., D. M. Kovacks, et al. (1996). "The presenilin genes and their role in early-onset familial Alzheimer's disease." Alzheimer's Disease Review **1**: 90-98.

Terry, R. D. (1996). "The pathogenesis of Alzheimer disease: an alternative to the amyloid hypothesis." The Journal of Neuropathology and Experimental Neurology **55**: 1023-1025.

Terry, R. D. (1963). "The fine structure of neurofibrillary tangles in Alzheimer's disease." The Journal of Neuropathology and Experimental Neurology **46**: 456-584.

Trojanowski, J. Q., M. Mawal-Dewan, et al. (1993). "Localisation of the mitogen activated protein kinase ERK2 in Alzheimer's Disease neurofibrillary tangles and senile plaque neurites." Brain Research **618**: 333-337.

Ulus, I. H. and R. J. Wurtman (1996). "Metabotropic glutamate receptor agonists increase release of slouble amyloid precurosr protein derivatives from rat brain cortical and hippocampal slices." The Journal of Pharmacology and Experimental Therapeutics **281**: 149-154.

Waite, J., J. M. Cole, et al. (1992). "Solvent effects on β-protein toxicity *in vivo.*" Neurobiology of Aging **13**: 595-599.

Walter, J., A. Capell, et al. (1997). "Ectodomain phosphorylation of β-amyloid precursor protein at two distinct cellular locations." The Journal of Biological Chemistry **272**: 1896-18903.

Wischik, C. M., M. Novak, et al. (1988). "Isolation of a fragment of tau derived from the core of the paired helical filament of Alzheimer's disease." Proceedings of the National Academy of the Sciences USA **85**: 4506-4510.

Wisniewski, T. and B. Frangione (1992). "Apolipoprotein E: a pathological chaperone protein in patients with cerbral and systemic amyloid." Neuroscience Letters **135**: 235-238.

Wisniewski, H. M., J. A. Sturman, et al. (1982). "Chronic model of neurofibrillary changes induced in mature rabbits by metallic aluminium." Neurobiology of Aging **3**: 11-22.

Wisniewski, H. M. and J. Weigel (1991). "Spatial relationships between astrocytes and classical plaque components." Nature Genetics **12**: 593-600.

Wolozin, B., K. Iwasaki, et al. (1996). "Participation of presenilin 2 in apoptosis: enhanced basal activity conferred by an Alzheimer mutation." Science **274**: 1710-1713.

Wong, P. C., H. Zheng, et al. (1997). "Presenilin 1 is required for *Notch*1 and *Dll*1 expression in the paraxial mesoderm." Nature **387**: 288-292.

Yanker, B. A. (1996). "Mechanisms of neuronal degeneration in Alzheimer's disease." Neuron **16**: 921-932.

Young, L. T., S. J. Kish, et al. (1988). "Decreased brain [^{3}H]inositol 1,4,5-trisphosphate binding in Alzheimer's disease." Neuroscience Letters **94**: 198-202.

Younkin, S. (1995). "Evidence that Aβ42 is the real culprit in Alzheimer's disease." Annals of Neurology **37**: 287-288.