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Effect of amyloid beta-peptide on synaptic plasticity in the Hippocampus in *vitro*

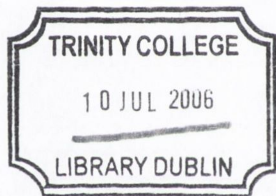
By

Qinwen Wang

A dissertation submitted for the degree of Doctor of Philosophy at the University of Dublin , Trinity College.

This research was carried out in the Department of Physiology.

September 2004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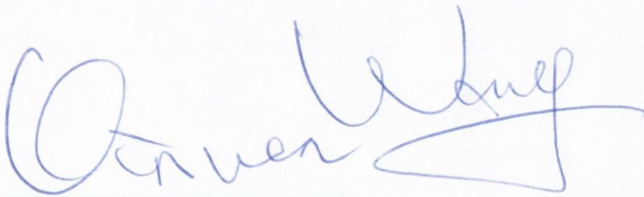

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Publications

Papers

1. Wang Q, Walsh DM, Rowan MJ, Selkoe DJ, Anwyl R.

Block of long-term potentiation by naturally secreted and synthetic amyloid beta-peptide in hippocampal slices is mediated via activation of the kinases c-Jun N-terminal kinase, cyclin-dependent kinase 5, and p38 mitogen-activated protein kinase as well as metabotropic glutamate receptor type 5.

J Neurosci. 2004 Mar 31;24(13):3370-8.

2. **Wang Q, Rowan MJ, Anwyl R.** Beta-amyloid-mediated inhibition of NMDA receptor-dependent long-term potentiation induction involves activation of microglia and stimulation of inducible nitric oxide synthase and superoxide.

J Neurosci. July 7, 2004, 24(27):6049-6056

3. **Marcus A. Westerman, Qinwen Wang, Linda A. Kotilinek, Giselle P. Lim, Agnieszka Falinska, Agnes Simonyi, Linda H. Younkin, Steven G. Younkin, Grace Y. Sun, Michael Rowan, James Cleary, Sally A. Frautschy, Greg M.Cole, Roger Anwyl, Karen H. Ashe.** NSAIDs improve amyloid β protein-induced deficits in memory and synaptic plasticity .

Neuron. Submitted

4. **Michael J. Rowan, Igor Klyubin, Qinwen Wang and Roger Anwyl.** Mechanisms of the inhibitory effects of amyloid beta-protein on synaptic plasticity.

Exp Gerontol. 2004 Nov-Dec;39(11-12):1661-7

5. Michael J. Rowan, Igor Klyubin, Qinwen Wang and Roger Anwyl.
Synaptic plasticity disruption by amyloid beta protein: modulation by potential Alzheimer's disease modifying therapies.
Biochem Soc Trans. 2005 Aug;33(Pt 4):563-7.

6. **Qinwen Wang, Jinqun Wu, Michael J. Rowan and Roger Anwyl.**
The□beta-amyloid inhibition of LTP is mediated via tumor necrosis factor.
European Journal of Neuroscience. Accepted

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Abbreviations

AD	Alzheimer's disease
Aβ	amyloid β peptide
APP	β-amyloid precursor protein
PF	protofibril
ADDL	Aβ-derived diffusible ligand
NFT	neurofibrillary tangle
PHF	paired helical filaments
TMD	transmembrane domain
PS	presenilin
LTP	long-term potentiation
E-LTP	early-phase LTP
L-LTP	Late-phase LTP
LTD	long-term depression
fEPSP	field excitatory postsynaptic potentials
EPSC	excitatory postsynaptic current
HFS	high frequency stimulation
LFS	low frequency stimulation
BDNF	brain- derived neurotrophic factor
CREB	cAMP response element binding protein
MAPK	mitogen-activated protein kinase
ERK	extracellular signal-regulated kinase
JNK	c-jun N-terminal kinase
SAPK	stress-activated protein kinase
PTK	protein tyrosine kinase
PKC	protein kinase C
PKA	protein kinase A
Cdk	cyclin-dependent protein kinase

CaMKII	calcium/calmodulin kinase II
GABA	gamma-aminobutyric acid
NMDA	N-methyl-D-aspartic acid
AMPA	α-amino-3-hydroxy-5-methyl-isoxazole-4-propionic acid
KA	kainate
mAChR	muscarinic acetylcholine receptor
mGluR	metabotropic glutamate receptor
nAChR	nicotine acetylcholine receptor
AChE	achacetylcholinesterase
ROS	reactive oxygen species
COX	cyclooxygenase
NO	nitric oxide
iNOS	inducible nitric oxide synthase
SOD	superoxide dismutase
TNF-α	tumor necrosis factor-alpha
IL	interleukin
NF-κB	nuclear factor κ B;
NSAID	non-steroidal anti-inflammatory drug
AA	arachidonic acid
PG	prostaglandin
CHO	Chinese hamster ovary
CM	conditioned medium

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Summary

Alzheimer's disease, the cause of one of the most common types of dementia, is a brain disorder affecting the elderly and is characterized by the formation of two main protein aggregates: senile plaques and neurofibrillary tangles. The pathogenesis mechanism of AD is not yet established. However, Several lines of evidence indicate that AD may be due, at least in part, the accelerated deposition of amyloid β–peptide(Aβ), which constitute a principal component of senile plaques.

The mechanisms of action of human synthetic and naturally secreted cell-derived amyloid β–peptide$_{1\text{-}42}$ (Aβ$_{1\text{-}42}$) on the induction of LTP were investigated in the medial perforant path to dentate granule cell synapse in rat and mouse hippocampal slices in vitro.

Synthetic and cell-derived Aβ strongly inhibited high frequency stimulation (HFS)-induced NMDAR-dependent LTP at peak and 1 hr post-HFS, although not induction of NMDAR-independent LTP or LTD. Cell-derived Aβ was much more potent than synthetic Aβ at inhibiting LTP induction, with threshold concentrations of ~1 nM and 100-200 nM respectively.

The involvement of various kinases in Aβ-mediated inhibition of LTP induction was investigated by applying Aβ in the presence of inhibitors of these kinases. The c-Jun N-terminal kinase (JNK) inhibitor JNKI prevented the block of LTP induction by both synthetic and cell-derived Aβ. The block of LTP induced by synthetic Aβ was also prevented by the JNK inhibitor SP600125, the Cdk5 inhibitors butyrolactone and roscovitine, and the p38 MAP kinase inhibitor SB203580, but not by the p42/44 MAP kinase inhibitor UO126.

The group I/group II metabotropic glutamate receptors (mGluR) antagonist LY341495 and the mGluR5 antagonist MPEP prevented the block of LTP induction by Aβ.

The α7 nicotinic receptor (nAChR) antagonist MLA did not prevent the inhibition of LTP induction by Aβ.

Evidence is presented in this study that the Aβ-mediated inhibition of LTP induction involves activation of microglia and production of reactive oxygen and nitrogen species. The inhibition of NMDAR-dependent LTP was prevented by minocycline, an agent that prevents activation of microglia.

The involvement of inducible nitric oxide (iNOS) was shown by the inability of Aβ to inhibit LTP induction in iNOS knockout mice, and also by the ability of two iNOS inhibitors, aminoguanidine and 1400W, to prevent the Aβ-mediated inhibition of LTP induction.

The Aβ-mediated inhibition of LTP induction was also prevented by the superoxide scavenger superoxide dismutase applied together with catalase. Evidence for involvement of superoxide in the action of Aβ on LTP induction was shown by the ability of an inhibitor of NADPH oxidase to prevent the Aβ-mediated inhibition of LTP induction.

The data demonstrate that A β causes inhibition of LTP induction by a series of events. We suggest that the Aβ-mediated inhibition of LTP induction is mediated via activation of mGluR5 and the kinases JNK, Cdk5 and p38 MAP kinase. The study also provides evidence that the Aβ-mediated inhibition of LTP induction involves an inflammatory-type reaction in which activation of microglia results in production of nitric oxide and superoxide and thence possibly peroxynitrite, a highly reactive oxidant.

1. Introduction

1.1 Preface

Alzheimer's disease (AD) is the most common form of degenerative dementia of human central nervous system (CNS) in the elderly (Katman et al., 1986; Francis et al., 1999; Perry and Hodges 1999, Selkoe 2001), which affects up to 15% of people over 65 years and nearly half of people whose age is older than 85 years(Smith et al., 1998). AD can be characterized clinically by a progressive impairment in cognitive function during mid- to late-adult life with the initial symptoms typically being certain forms of memory and language losses (Selkoe et al., 1997,2004; Lawrence and Sahakian 1998, Bozeat et al., 2000; Marin et al., 2002). The major neuropathological changes in brains of AD patients were observed first by Alois Alzheimer (1907) and include neuronal cell death accompanied the presence of abnormal intra- and extraneuronal proteinaceous deposits (Katzman, et al., 1986). Intracellularly, formation of neurofibrillary tangles (NFTs) composed of paired helical filaments (PHFs), composed of hyperphosphorylated tau (Goedert M, 1993; Kosik, 1991). Extracellularly, amorphous insoluble aggregates of proteinaceous debris termed "amyloid" appear in the form of senile plaques or neuritic plaques and cerebrovascular amyloid deposits (Glenner, 1980).

There is no cure for AD, and its molecular mechanism is not yet established. However, lines of evidence suggest that the amyloid β peptide (Aβ), the major component of plaques, a 4.1-4.3kD cleavage product of β-amyloid precursor protein (APP) plays a critical role in AD.

1.2 Origin and structure of Aβ

1.2.1 Biogenesis of A β

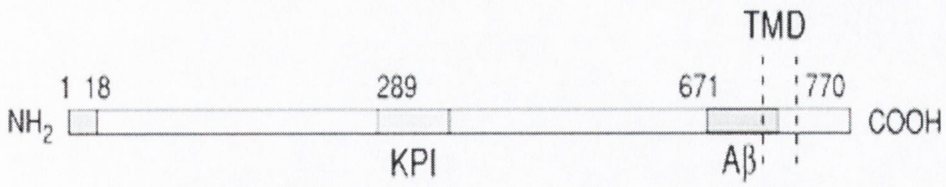
The purification and sequencing of the Aβ amyloid deposits in AD and Down's syndrome (Glenner and Wong, 1984) and the subsequent discovery that Aβ was the subunit of the plaque of amyloid (Master et al., 1985; Gorevic et al., 1986; Selkoe, et al., 1986) led to the cloning of gene encoding APP (Kang et al., 1987). Now we know Aβ is part of a larger amyloid precurer protein, it is a small hydrophobic peptide with N- and C-terminal heterogeneity that occurs in two principal lengths, Aβ40 and Aβ42.

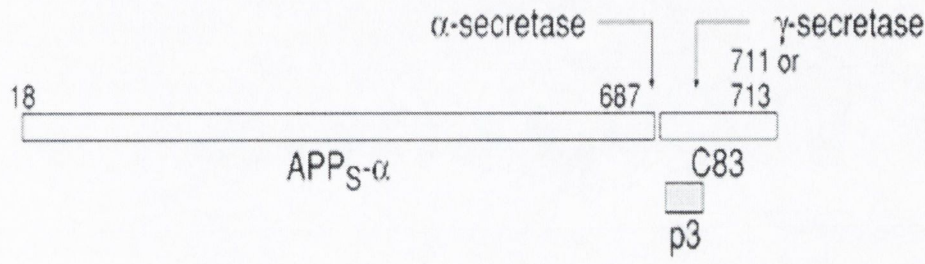
APP has 3 major isoforms of 695, 751 and 770 residues, these isoforms are derived from alternatively spliced transcripts encoded by a single gene on chromosome 21 at 21q21.2 (Robakis et al., 1987; Kang et al., 1987; Tanzi et al., 1987). The gene has 18 exons of which exons 16 and 17 encode in part for Aβ (Glenner and Wong, 1984). APP containing 751 and 770 residues are widely expressed in non-neuronal cells and also

occur in neurons. The isoform of 695 amino acid has a higher expression level in neurons than that in non-neurons (Haass et al., 1991).

APP is a single transmembrane polypeptide that is co-translationally translocated into the endoplasmic reticulum via its signal peptide and then post-translationally modified through the secretory pathway (Weidemann et al. 1989). APP can undergo a sequential proteolytic cleavage by α- , β- and γ- secretases and then release the derivatives into vesicle lumens and extracelluar space. The Aβ region of APP comprises the 28 residues just outside the transmembrane domain (TMD), plus the first 12-14 residues of that buried domain (Fig.1).

Most APP molecules are cleaved by α- secretase, rather than β-secretase. The common cut occurs between amino acids 16 and 17 of the β-amyliod protein region, that is, 12 residues N-terminal to the transmembrane region and is carried out by α-seretase. This scission creates a large, soluble ectodomain fragment (APP α) that is released from the cell surface and leaves a C-terminal fragment of 83 amino acids (C83 or α-CTF) still embedded in the membrane. Some APP molecules that fail to proteolytic processing by a-secretase can be internalized into endocytic compartments and subsequently cleaved between Met671 and Asp672 of APP by β-secretase (BACE) which is a membrane-anchored aspartyl protease with its active site in its ectodomain. A slightly shorter form of APP β is released from cell surface and a C-terminal





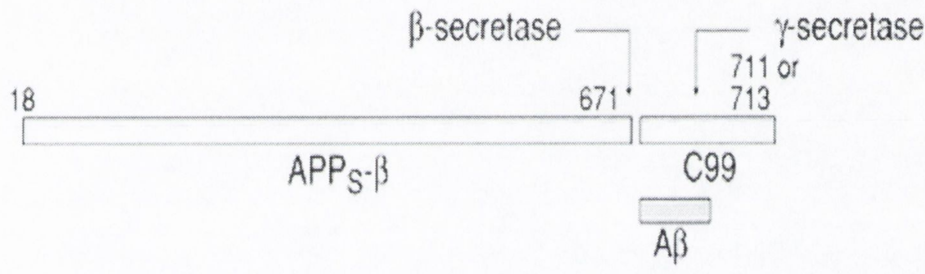


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Fig1.1 Schematic diagrams of the APP and its principal metabolic derivatives. The first line depicts the largest of the known APP alternate splice forms, comprising 770 amino acids. Regions of interest are indicated at their correct relative positions. A 17-residue signal peptide occurs at the *N*-terminus (box with vertical lines). Two alternatively spliced exons of 56 and 19 amino acids are inserted at residue 289; the first contains a serine protease inhibitor domain of the Kunitz type (*KPI*). A single membrane-spanning domain (transmembrane [*TM*]) at amino acids 700 through 723 is indicated (dotted lines). Aβ fragment includes 28 residues just outside the membrane plus the first 12 to 14 residues of the TM domain. In the second line, the sequence within APP that contains the β-amyloid protein and TM regions is expanded. The underlined residues represent the β-amyloid proteins 1 to 42 peptide. The letters below the wild-type sequence indicate the currently known missense mutations identified in certain families with AD or hereditary cerebral hemorrhage with amyloidosis. The 3-digit numbers are codon numbers (APP 770 isoform). In the third line, the first arrow indicates the site (after residue 687) of a cleavage by β-secretase that enables secretion of the large, soluble ectodomain of β-amyloid precursor protein (APP_s-β) into the medium and retention of the 83-residue C-terminal fragment (*C83*) in the membrane. The C83 fragment can undergo cleavage by the protease called γ-secretase at residue 711 or residue 713 to release the p3 peptides. The fourth line depicts the alternative proteolytic cleavage after residue 671 by β-secretase that causes the secretion of the slightly truncated APP_s-β molecule and the retention of a 99 residue C-terminal fragment (*C99*). The C99 fragment can also undergo cleavage by γ-secretase to release the Aβ. Cleavage of both C83 and C99 by γ-secretase releases the β-amyloid precursor protein intracellular domain into the cytoplasm.

fragment of 99 amino acids (C99 or β-CTF) that can be subsequently be cleaved by γ-secretase to create A β and γ-CTF. The C83 also undergo cleavage by the same γ-secretase to generate a small peptide called as p3.

The major of secreted Aβ peptides are 40 amino acids in length (Aβ 40), although the smaller fraction of longer 42 amino acids species have received greater attention due to the propensity of these peptides to nucleate and drive production of amyloid fibrils (Jarrett et al., 1993)

1.2.2 Assemblies of A β and A β neurotoxicity hypothesis

1.2.2.1 Structure base of A β aggregation

The complete sequence of Aβ was described by Kang et al.(1987). Residues 28-42 of Aβ, which correspond to its C terminus, are embedded in the plasma membrane, and the sequence is rich in the hydrophobic amino acids valine and isoleucine. Leucine and valine are often found in proteins with β-sheet structure and rarely found in helical or random coil secondary structures (Chou and Fasman, 1978). Furthermore, the presence of glycine residues can stabilize amyloidogenic structures by means of hydrogen bonds (Lansbury, et al., 1992). Studies also indicated a great hydrophobicity in the last 10 amino acids at the C terminus of Aβ, as well in the sequence between residues 17 and 21. Chou-Fasman's analysis suggests the existence of α-helix, β-turn, and β-strand types in the secondary structures of Aβ. The probability of finding β-strand

conformation in Aβ is high within the C-terminal region beyond residue 28; the probability is lower between residues 9 and 21. The latter region is more likely to display α-helix structure. There are also two probable β-turns between residues 6 and 8 and between residues 23 and 27. A model for the tri-dimensional structure of Aβ in its soluble form was built. The theoretical structure of Aβ consists of two β-strands separated by a turn that form a small α-helix. This structure could be the basic unit for fibril formation through hydrogen bonding between the N-terminal β-strand of one peptide and the C-terminal β-strand of the other peptide.

Using synthetic peptides, studies suggest that sequences 1-28, 12-28, and 14-28 lead to amyloid fibril formation in vitro (Gorevic et al., 1987), and that the markers of β-pleated conformation are present in a minimum stretch of 15 residues (14 to 28). The following hydrophobic domain proved unnecessary for fibril formation, but may help make Aβ more insoluble (Gorevic et al., 1987). In fact, it has been shown that the C terminus of Aβ determines the rate of amyloid fibril formation rather than the stability and structural properties of the amyloid (Jarrett et al., 1993).

The substitution of lysine 16 for an arginine in the fragment 1-28 gives rise to a morphologically different fibril that retains its β-sheet structure. Nevertheless, the x-ray diffraction pattern suggests that this fibril contains a larger number of β-pleated sheets in the same fibril diameter (Kirschner et al., 1987). The substitution of valine 18 (an amino

acid-forming β-sheet) for an alanine (an amino acid-forming α-helix) on the Aβ aggregation. The modified peptide becomes significantly less aggregated than Aβ unmodified (Soto et al., 1995).

The substitution of glutamate 22 by glutamine (Levy et al., 1990), yields a peptide having an increased ability to form fibrils (Wisniewski et al., 1991). This peptide generates fibrillar structures in 1 h compared with the 24 h necessary for wild-type peptide. On the other hand, the replacement of hydrophobic for hydrophilic residues in the Aβ sequence impairs the formation of fibrils (Hilbich et al., 1991). This suggests that the insolubility of Aβ is mainly due to its hydrophobic residues. The same idea is supported by studies using synthetic peptides of the C-terminal side of Aβ, namely, residues 26-33 and 34-42 (Halverson et al., 1990). Interesting differences emerged in their solubility and conformational properties. For example, peptide 26-33 was freely soluble in water, whereas peptide 34-42 was insoluble in aqueous media and also in the presence of denaturating agents. The peptide 26-33, when dissolved in water, existed as a random coil, whereas the water-insoluble peptide 34-42 possessed antiparallel β-sheet structure in the solid state. Solubilization of peptide 34-42 in organic media resulted in the disappearance of β-structure (Halverson et al., 1990).

Elements of the extracellular matrix have been also suggested to play a role in the aggregation of Aβ (Fraser et al., 1992; Brandan and

Inestrosa, 1993). Sulphate ions have been revealed the specific influence at concentrations between 5 and 50 mM in the extensive lateral aggregation and axial growth of the synthetic peptides into 'macrofibers' (Fraser et al., 1992). This suggests that sulphate is required to promote aggregation. Highly sulfated molecules within the extracellular matrix, such as proteoglycans, may provide a high-affinity surface for the direct deposition of existing amyloid fibrils at the extracellular matrix of brain tissue (Brandan and Inestrosa, 1993).

Barrow and Zagorski (1991) have reported that local variations in pH or temperature, could induce the aggregation of Aβ. They found fragment 1-28 was found as a monomeric α-helical structure when solubilized in a membrane-mimicking solvent. This soluble peptide unfolds to a partly random coil structure with increasing temperature at pH 1-4 and pH greater than 7. However, at pH 4-7 it rapidly precipitates into an oligomeric β -sheet structure. Nevertheless, the hydrophobic segment in the C-terminal domain of Aβ (residues 29-42) invariably adopts an oligomeric β-strand structure, independent of the pH or temperature, suggesting that this segment directs the complete protein folding (Barrow and Zagorski, 1991; Barrow et al., 1992). Thus, the first 28 residues, which are strongly dependent on factors such as the hydrophobicity and pH of the environment, enable Aβ to exist in different conformations. The change in structure from an α-helix to a β-pleated

sheet corresponding with the change from acidic to midrange pH has been modeled (Zagorski and Barrow, 1992). The model is based on the presence of ionizable groups, such as the side chains of aspartic and glutamic acids, present in the conformational neighboring residues of the α-helix : amino acids 7, 11, and 22.

Overall, Aβ would contain two conformational domains: the C-terminal segment (amino acids 28-42) is incorporated within the plasma membrane and always exists in solution as a β-strand; the N-terminal domain adopts alternative secondary structures, depending on pH, hydrophobicity, and the presence of extracellular matrix components. With these alternative structures, Aβ can adopt two distinct conformations with different solubility properties, which could explain why the same amino acid sequence can exist either in dissolved or aggregated forms.

1.2.2.2 Assemblies and solubility of Aβ

The Aβ peptide is recognized for its self-aggregation into amyloid (Harper and Lansbury 1997, Masters and Simms, 1985). Amyloid comprises large fibrils and β-sheet secondary structure, which is characterized by Congo red or thioflavin S staining. Similar fibrils assemble in vitro from synthetic peptide (Jarrett et al., 1993). Aβ can

exist in a variety of different forms, up to now, the following assembly forms have been reported: monomers; SDS-stable low-n oligomer; Aβ-derived diffusible ligand (ADDL); protofibrils (PF) and fibrils.(Lue et al., 1999; McLean, et al., 1999; Wang et al., 1999; Kuo et al., 1996). They usually are divided into two groups, soluble and insoluble forms. The term soluble Aβ is an operational definition, embracing all forms of Aβ that remain in aqueous solution following high- speed centrifugation of physiological buffer extracts of brain.

In aqueous solution, Aβ shows only a limited solubility at a lower concentration, containing essentially monomers with random coil structure. Solutions with higher concentrations exhibit a reversible, concentration-dependent equilibrium between random monomers and β-structure aggregates(Teri et al 1995). Under physiological pH only a part of Aβ became aggregated even at long incubation times and high peptide concentration (Barrow, et al., 1992; Burdick , et al., 1992; Bush, et al., 1994), in fact, the sedimentable peptide reaches a maximum around 40% after 3 days of incubation, which remains unaltered even after 14 days.

The solubility of Aβ was shown to be pH and concentration –dependent (Barrow and Zagorski, 1991; Barrow et al, 1992). An equilibrium in solution between solubilized and insoluble aggregated peptide fraction was observed (Hilbich et al. 1991). Other studies also

suggested an equilibrium between amyloid deposition and fibril dissociation(Hyman et al., 1993; Tamaoka et al., 1994).

1.2.2.3 Neurotoxicity of insoluble(fibrils) A β

Early studies supported the hypothesis that fibrils of Aβ derived neurodegeneration in AD (Hardy and Higgins 1992). Studies over many years had determined that Aβ forms insoluble aggregates and fibrils that are the major component of the spheroid plaque structure with which degenerating neurons are often associated (Selkoe, 1993). Cell culture studies have shown that exposure of solutions containing large fibrils killed cultured neurons whereas solutions of monomer were harmless (Lorenzo and Yankner 1994; Pike et al., 1993). Studies also suggests that the development of neurotoxicity of Aβ is related to the fibrillar state of the peptide (Howlett, et al 1995). Other studies linked inherited, early-onset AD to various mutations in APP and in presenilins 1 and 2 (PS1 and PS2) that increase production of highly fibrillogenic Aβ (Selkoe 1997, Scheuner et al.1996). These evidence has implicated amyloid fibril induced neuron death as a primary cause of AD.

The problem of the hypothesis is the poor correlation between fibrillar amyloid load and measures of neurological dynfunction. In AD, amyloid deposits often form at a distance from sites of neuron loss,

moreover, they also develop in cognitively normal individuals who have no local neuron damage. Transgenic mice research for AD also reported that most of the neurodegeneration occurs in the absence of amyloid deposits (Mucke et al., 2000).

Recently, the profound disconnection between pathogenesis and amyloid led the amyloid cascade hypothesis modified to include additional pathogenic Aβ assemblies, which are quite different in structure from amyloid fibrils(Hardy and Selkoe 2002, Klein et al 2001).Evidence suggested Aβ toxicity is likely to be mediated by multiple different Aβ assembly forms, soluble and fibril Aβ.

1.2.2.4 Neurotoxicity of soluble(non-fibril) Aβ

The recent studies show the neurotoxicity can be fibril-independent. In two studies (Dodart et al., 2002, Lambert et al., 1998), memory failure in human APP (hAPP) mice was actually reversed by Aβ antibodies without reduction in amyloid plaque level. Another report showed recovery was observed in plaque filled mice by a single injection of Aβ antibody. They are consist with the first evidence (Oda et al., 1995) from the experiments in which ApoJ inhibited the formation of Aβ fibril, but did not inhibit Aβ neurotoxicity.

Solutions of monomeric Aβ are at first innocuous but with time develop neurotoxicity, it still need self-aggregation (Pike, et al., 1993; Lorenzo, et al., 1994; Howlett, et al., 1995).

Aβ dimers activate glial cells and can lead to nerve cell death in co-cultures containing astrocytes, but these dimers have no direct neurotoxicity (Roher, et al., 1996). Direct action on neurons is evident, however, with preparations of PFs, which rapidly induce action potentials and other electrophysiological responses, and, with longer exposure, cause cell death (Walsh, et al., 1999; Hartley et al., 1999)

One neurotoxic form of Aβ is ADDL which is non-fibrillar. Unlike fibrils, their structure comprises relatively small globular oligomers ranging from trimers to 24mers. (Lambert et al., 1998). ADDLs are more stable than PFs at low levels of Aβ, particularly for the AD-linked Aβ species. Metabolically derived oligomers accumulate in conditioned cell culture medium without evidence of PFs or amyloid fibrils (Podlisny, 1995). Oligomer levels increase after transfection with mutated familial AD presenilins (Xia et al 1997), which elevate levels of Aβ. Solutions of synthetic Aβ oligomers without fibrils or PFs can be made readily (Lambert , et al., 1995), although the converse is not true. The stability of fibril-free solutions of toxic oligomers is consistent with the plaque-free pathology of transgenic mice discussed above.

Another toxic form is the Aβ-derived protofibril, initially discovered as an intermediate in Aβ1-40 amyloidogenesis (Walsh et al.,1999). By atomic force microscopy, PFs can be seen to be curvilinear structures of 4-11 nm diameter and <200 nm length (Hartley et al.,1999). In others reports, PFs are the largest of the subfibrillar toxins, ranging to 400 nm in length and 1000 000 Da in mass (Walsh, et al., 1997 and Harper et al., 1997). PFs are large rod-shaped molecules different in structure from ADDLs. Theoretically, the smaller ADDLs, which are globular molecules, might serve as precursors to the rod-shaped PFs. Electron microscopy experiments have suggested, moreover, that a typical PF preparation might also contain ADDLs, and they appear to be intermediates on the pathway to amyloid fibril formation (Walsh, et al., 1997, Harper, et al., 1997).

Unlike ADDLs which form readily even at low nM concentrations that could be attainable in vivo, the lower limit has not been determined (Chang et al., 2001), PF formation significantly require higher levels of Aβ monomer. They lack stability at low monomer concentrations but nonetheless can be isolated by molecular sieve chromatography. Isolated PFs, although free of monomers and amyloid fibrils, are bioactive. In cell culture, PFs cause oxidative stress and, eventually, neuronal death (Walsh et al.,1999). They also elicit rapid electrophysiological changes, inducing

membrane depolarization, and increased EPSPs and action potentials (Hartley et al.,1999). In spite of their thermodynamic instability, PFs apparently occur in CSF, according to a preliminary analysis of individuals with AD (Pitschke et al., 1998).

1.3 Aβ and synaptic plasticity

1.3.1 Long-term potentiation

One form of activity-dependent synaptic plasticity that has been investigated extensively is long-term potentiation (LTP) which is believed to represent one of the cellular mechanism for learning and memory. LTP is an artificially induced phenomenon, when excitatory synapses are electrically stimulated at high frequency for brief period, for example 100 Hz for 1 second, the strength of those synapses increases, which can persist for many weeks (for hours in hippocampal slices in vitro and even months in vivo).

LTP occurs in many pathways of the brain (Martin, et al., 2000), like amygdala and cortex, not just the hippocampus and dentate gyrus, where it was first observed in the hippocampus of rabbits in vivo (Bliss and Lømo in 1973, Bliss & Gardner-Medwin, 1973).

In the hippocampus, all three main pathways , perforant path, the mossy fibres and Schaffer Collateral-Commissural pathways, generate

LTP by HFS stimulation. Pharmacological experiments have identified the different aspects of LTP, referred as induction, maintenance and expression.

1.3.1.1 Characteristics of LTP

LTP is characterized by the properties of input-specificity, cooperativity and associativity. Input-specificity means that only those inputs activated at the time of the tetanization will display LTP (Anderson et al., 1977 and Lynch et al., 1977). Co-operativity refers to the existence of a certain intensity threshold for the generation of LTP; weak tetanic activation of a relatively small number of afferents is filtered out. Thus, a number of afferent fibres must be active at the same time, and for a minimum period of stimulation, if a long-lasting potentiation is to be induced; stimulation of only a few fibres is not sufficient to induce LTP (Bliss and Lynch, 1988). Consequently, induction requires association between the pre- and postsynaptic events (presynaptic activation and postsynaptic depolarisation), and only synapses active inside a certain time window with respect to the postsynaptic activity are potentiated (Gustafsson, et al., 1990). Associativity means that a weakly tetanised input can express LTP when other independent afferent fibres,

converging to the same target cells, are simultaneously stimulated by a strong tetanus (Voronin et al., 1995).

1.3.1.2 The role of glutamate receptors in LTP

Glutamate receptors are either ionotropic ligand gated ion channels or metabotropic. In metabotropic group, the signal is transduced to other intracellular messengers like inositol trisphosphate or cyclic AMP. The ionotropic glutamate receptors are further classified, according to their interactions with non-physiological glutamate analogs, to NMDA (N - methyl-D-aspartate), AMPA (amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid) and KA (kainate) receptors, the latter two often being denominated together as non-NMDA receptors. It is well established that the glutamate receptors play a critical role in the neuronal survival of neuronal connections during brain development, as well as in the synaptic plasticity underlying learning and memory.

1.3.1.3 The role of NMDA receptors in LTP induction

NMDA receptor is composed of 7 subunits- NR1, NR2A-D and NR3A-B. Five separate genes encodes for the first 5 different subunits, and recently NR3A and NR3B were identified (Chatterton et al., 2002;

Belenikin et al.; Seeburg, et al, 1993). NR1 can be divided into a large N-terminal region, a core region including four TMDs, and C-terminal extension. It is generally agreed that the N-terminal region is extracellular and C-terminal region is intracellular. NR1 seems to be essential for function and requires co-expression with at least one of other subunits to form NMDA receptors. The fundamental properties of NMDA receptor such as voltage dependent of Mg^{2+} block, the Ca^{2+} permeability, and regulation by phosphorylation and channel kinetics are modulated by NR2A, NR2B, NR2C, and NR2D subunits.

In contrast to AMPA/KA subtypes of glutamate-gated ion channels which open and desensitize rapidly in response to an agonist, and selectively permit influx of Na^{+}, but not Ca^{2+}, the NMDA receptors allow influx of both Na^{+} and Ca^{2+} from an extracellular environment, and their activation/deactivation kinetics are slow. NMDA receptors are cellular coincidence detectors and require simultaneous binding of glutamate and depolarization in order to remove the voltage-dependent Mg^{2+} block from the channel (Ascher and Nowak, 1988).

In condition of normal synaptic transmission (insufficient to elicit LTP), glutamate binds both AMPA and NMDA receptors, but since the majority of NMDA receptors are voltage blocked by the extracellular Mg^{2+} , the EPSP is mainly mediated by the AMPA receptor.

During a tetanic stimulation there is a strong and sustained depolarization of cell due to the activation of AMPA receptors. AMPA receptors activation is eventually sufficient to relieve the voltage-dependent block of Mg on NMDA receptors and release of glutamate by a strong tetanus is sufficient to fully activate NMDA receptors. Since NMDA receptors are highly permeable for Ca^{2+}, and this increase in postsynaptic Ca^{2+} appears to play a critical part in the activation of further intracellular processes which may constitute LTP.

Thus NMDA receptors are essential for triggering of LTP. Activation of NMDA receptors appears to be crucial for LTP at most synapses (Collingrdige et al., 1983, Bliss and Collingrdige 1993).

1.3.1.4 The role of mGluRs in LTP and NMDA-independent LTP

NMDAR is not the only glutamate receptor that participates in the induction of long-lasting plasticity. mGluRs are also involved in the induction of long-term synaptic plasticity in the hippocampus, cortex and cerebellum, but evidence for its contribution in the induction of long-lasting plasticity is more recent. In CA1 LTP may not depend on NMDA receptor activation if tetanic stimulation is stronger than the conventionally used 100 Hz (Grover and Teyler, 1990). This NMDA-independent LTP requires activation of voltage-dependent Ca^{2+}

channels. The exact nature of this form of potentiation awaits further investigations.

mGluRs can be subdivided into three groups based on the homology of their amino acid sequences, group I mGluR, group II mGluR and group III mGluR (Nakanishi, 1992; Pin and Duvoisin, 1995). Group I mGluR comprises mGluR1 and mGluR5. Group I mGluRs are positively coupled to phospholipase(PLC) which in turn leads to hydrolysis of phosphoinositide (PI) and the production of IP3 and DAG. Group II mGluRs consist of mGluR2 and mGluR3 and are negatively coupled to adenylycyclase. Group III mGluRs consist of mGluR4, mGluR6, mGluR7 and mGluR8 and are also negatively coupled to adenylycyclase (Suzdak et al., 1994; Pin and Duvoisin, 1995). mGluRs are a class of G-protein coupled receptors which possess seven putative transmembrane regions. mGluRs have a cysteine-rich N-terminal extracelluar domain where the agonist binding site is located (O' Hara et al., 1993; Okamoto et al., 1998; Han and Hampson, 1999; Peltekova et al., 2000). The C-terminal domain of mGluRs is variable in length.

There is considerable debate surrounding the role of mGluRs in LTP. Initially, the involvement of metabotropic receptors in LIP was demonstrated in hippocampal CA1 in slice experiments using L-AP4 as an antagonist (Reymann and Matthies, 1989) which inhibited LTP. This

observation has been confirmed by use of GAP3 (Behnisch et al., 1991; Izumi et al., 1991; Behnisch and Reymann, 1993).

Many studies have reported that MCPG, an antagonist of group I mGluRs and group II mGluRs, blocks the inhibition of LTP (Bashir et al., 1993; Breakwell et al., 1998; Fitzjohn et al., 1998; Grover et al., 1998; Wilsch et al., 1998). But some studies showed no effect of MCPG on LTP induction in CA1 and CA3 in vitro in some cases (Chine et al., 1993; Manzoni et al., 1994). ACPD, an agonist of group I and group II mGluRs, has been shown to facilitate and induce LTP in CA1 (Bortolotto and Collingridge, 1993, 1995; Breakwell et al., 1996 McGuinness et al., 1991). Bortolotto and Collingridge (1993, 1995) reported that it is NMDA receptor independent.

The role of mGluRs in LTP has been most extensively studied in CA1 synapse where mGluR5 is the most strongly expressed subtype (Shigemoto et al., 1997). Jia et al. reported (1998) a complete deficit of NMDA-receptor dependent component of LTP while AMPA-receptor mediated component of LTP was unaffected in mGluR5 knockout mice. In mGluR1 knockout mice, Aiba et al (1994) shown a deficit of LTP in CA1 and normal LTP in CA3 while in another study there was an unaffected LTP in CA1 and dentate gyrus and inhibited LTP in CA3 (Shigemoto et l., 1997).

Some groups reported that activation of group II mGluR might have an inhibitory effect on LTP by using ACPD and DCG-IV(Holscher et al 1997, Behnisch et al., 1998), and MOSSEPE, an antagonist of group II mGluR, has been found to enhance LTP (Behnisch et al., 1998). However, other studies have failed to replicate this ether in vivo or vitro. In a recent study, Wu et al shown a NMDA-independent LTP which is mediate by group II mGluR since the LTP was inhibited by the group mGluR antagonist EGLU and induced by an agonist, DCG-IV in media containing D-AP5.

Antagonists at group III mGluR in CA1 have failed to block the induction of LTP (Breakwell et al., 1998; Fitzjohn et al., 1998). L-AP4, agonist on presynaptically located group III mGluRs, caused depression of synaptic transmission of baseline responses in CA1 and dentate in young animals (Manahan-Vaughan and Reymann, 1995), but not in older rats. In animals of the latter group, however, tetanus-induced potentiation was reduced in amplitude and duration in the presence of L-AP4 returning to baseline after 15 min.

Taken together, the data suggest that activation of mGluRs may facilitate or depress the capability of synaptic plasticity, depending on subtypes activated, the data also indicate a modulatory role for mGluRs in LTP, especially when titanic conditions are weak, under which mGluR

activation may amplify and prolong incoming signals, and the block of mGluRs may render conditions for LTP induction subthreshold.

1.3.2 Long-term depression

LTD Long-term depression (LTD), as a counterpart to LTP, is a lasting decrease in synaptic efficacy. LTD can be induced in several brain regions, including hippocampal region CA1 (Levy et al., 1979), the dentate gyrus (Abraham et al., 1983 and Braham et al, 1987), visual cortex (Artola, et al., 1993), frontal cortex (Hirsch and Crepel, 1992) and cerebellum (Ito, 1989). LTD of can be categorized into homosynaptic and heterosynaptic LTD according to the pathway and the pattern (Braham et al, 1987; Artola, et al., 1993, Linden et al 1994). Heterosynaptic LTD can be produced by presynaptic activation of different inputs on to the same postsynaptic cell, but homosynaptic LTD can be induced by presynaptic activation of the same input on to the relevant neuron (Linden et al 1994). Several factors have been reported to trigger LTD, including the induction of LTP, depolarization of post-synaptic sites, activation of NMDA and non-NMDA receptors, activation of metabotropic glutamate receptors and moderate postsynaptic $Ca2+$ influx (Linden et al 1994, Kano et al 1987). Homosynaptic LTD has been found in a number of

brain structures using one of two induction protocols: prolonged afferent stimulation at low frequency (1–5 Hz for 5–15 min) or stimulation at high frequency (50–100 Hz for 1–5 s). Low-frequency stimulation has been shown to induce homosynaptic LTD in the hippocampus but high-frequency stimulation has been effective at striatal synapses (Braham et al, 1987, Lovinger, et al 1993). By contrast, induction of heterosynaptic LTD requires a strong postsynaptic depolarization caused by synaptic activation. It should be noted that heterosynaptic depression in area CA1 has proved difficult to investigate, as several groups have failed to induce this type of LTD at all (Linden et al 1994).

A novel type of LTD induced by muscimol, a $GABA_A$ agonist, has recently been reported. It is reversed by the $GABA_A$ antagonist bicuculline, and potentiated by neurosteroids such as alphaxalone, benzodiazepines and barbiturates, which are positive modulators of $GABA_A$ receptors (Akhondzadeh et al 1995; 1996; 1998). Moreover, prenenolone sulphate, a negative modulator of $GABA_A$ receptors, can block the induction of muscimol-induced LTD (Akhondzadeh et al 1998). It has also been reported that this type of LTD can be prevented by application of brain-derived neurotrophic factor (BDNF) and this phenomenon may explain the mechanism of muscimol-induced LTD through a bilateral relation between $GABA_A$ activity and BDNF (Akhondzadeh et al., 1999). In addition, the NMDA, non-NMDA and

metabotropic glutamate receptors are not involved in the induction of this type of LTD (Akhondzadeh et al 1996). The activation of synapses is not necessary for the induction of this LTD, as the long-lasting depression could be induced when there was no stimulation during perfusion of muscimol (Akhondzadeh et al., 1995).

1.3.3 Aβ and LTP

There is now considerable evidence that Aβ-induced dysfunction of synaptic plasticity contributes to early memory loss that precedes neuronal degeneration (Small, et al., 2001; Selkoe, 2002, Cullen et al, 1997; Lambert et al, 1998; Itoh et al, 1999; Chen et al, 2000; Stephan et al, 2001; Vitolo et al, 2002).

Several groups have reported that synthetic Aβ inhibits LTP induction in vitro and in vivo. Synthetic Aβ 1–42, Aβ 1–40, and the truncated Aβ fragment 25–35 were found to inhibit LTP induction of the medial perforant path in the dentate gyrus and CA1 slices. Both of the population spike (Lambert et al. 1998) and EPSPs (Wang et al. 2002), and both early- and late-phase LTP were strongly inhibited in these studies, whereas basal AMPA receptor mediated synaptic transmission was not altered, although there was a reduction in paired-pulse depression at a short (20 ms) inter-pulse interval (Wang et al. 2002). The N-terminal

sequence of Aβ 25–35 was found to be necessary for inhibition of LTP induction (Chen et al. 2000). In some studies, non-fibrillar Aβ 1–42 (Wang et al. 2002) and Aβ variants that did not form fibrils in vitro (Chen et al. 2000) selectively inhibited both short-term and long-term synaptic plasticity, indicating a critical role for soluble peptide.

In contrast to these studies showing an inhibition of LTP induction, synthetic Aβ 1–40 (200 nM) enhanced LTP induction in the associational–commissural pathway of the dentate gyrus of 30–50-day-old rats (Wu et al. 1995). Basal AMPA receptor-mediated synaptic transmission was not affected in this study. Because there are no changes of baseline transmission in these studies. the inhibitory effects of Aβ on LTP in vitro do not appear to be caused by a toxic action of the Aβ resulting in rapid neurodegeneration.

Consistent with most in vitro studies, synthetic Aβ also inhibits LTP in vivo. Thus late-phase LTP of field EPSPs in the CA1 area was strongly inhibited at doses that had no acute effect on baseline excitatory transmission in adult rats by intracerebroventricular i.c.v.) injection of Aβ 1–40 (0.4 and 3.5 nmol, but not 0.1 nmol), Aβ 1–42 (0.01 nmol) and the Aβ -containing C-terminal fragment CT105 (0.05 nmol). In these studies, LTP was only significantly inhibited at a time greater than 2 h post-HFS, and the LTP was completely blocked by Aβ 1–40 and Aβ 1–42 at 3 h post-HFS (Cullen et al, 1997), implicating late LTP. Somewhat similarly,

LTP of EPSPs in the CA1 area was inhibited by the truncated fragments Aβ 25–35 (10 nmol, 100 nmol) and Aβ 35–25, but not Aβ 15–25, at times greater than 30 min post HFS (Freir et al. 2001).

Other studies have examined the delayed neurophysiological effects of Aβ in vivo. In contrast to the acute effect of Aβ 1–40 (3.5 nmol), there was a small reduction in baseline transmission in the CA1 area 24 h after a single i.c.v. injection. The reduction was present for at least 5 days, whereas LTP was not affected at this time (Cullen et al. 1996).

In another study, induction of LTP of field EPSPs in the dentate gyrus by strong HFS was inhibited after direct intrahippocampal injection of Aβ 1–43 or a combination of Aβ 1–40 and Aβ 1–43 in adult rats. Late-phase LTP of the EPSP was most sensitive to disruption, whereas EPSP-spike LTP was largely intact. The effect of the Aβ was examined 7–16 weeks after the injections, a time when focal amyloid deposits and cell atrophy were detected. A reduction in baseline synaptic transmission and deficits in working memory were also present (Stephan et al. 2001).

Somewhat analogous to the in vitro studies on transgenic mice, two studies examined the effects of in vivo Aβ exposure on synaptic function in the hippocampal slice of adult rats. Whereas acute single i.c.v. injection of synthetic Aβ 1–40 (0.4 or 3.5 but not 0.1 nmol) caused a reduction in baseline transmission and no change in LTP in the dentate gyrus 48 h later (Cullen et al. 1996), continuous i.c.v. infusion of Aβ 1–

40 (300 pmol day21) for 10–11 days inhibited LTP of the population spike in the CA1 area (Itoh et al. 1999). In the latter study, there was a tendency to require a greater current to evoke equivalent-sized spikes.

In experiments with rat and mouse CNS tissue, ADDLs have been found to impair synaptic plasticity in the short-term and cause selective neuron death in the longer-term. In addition, indirect evidence suggests ADDLs may cause loss of synaptic terminals. In the short-term, ADDLs inhibit hippocampal LTP. Inhibition occurs within 45 min, and it is complete at submicromolar dose (Wang et al. 2002). ADDLs do not inhibit LTD, but interestingly, ADDLs do inhibit recovery from LTD (Wang et al., 2001). ADDLs thus shift synaptic plasticity in a negative direction, blocking LTP while reinforcing LTD. This negative shift ultimately may be related to a destabilization of synapses. The second neurological consequence of ADDLs appears to be synapse loss, suggested by the indirect evidence from transgenic-mice. ADDLs induce synaptosis, whereby synapses are destabilized and removed. ADDLs also inhibit synapse replacement. Such effects could be caused by localized action of ADDLs at synaptic terminals. The third neurological consequence of ADDLs is selective neuron death. Death occurs within several days and can occur at low ADDLs doses.

1.3.4 Aβ and LTD

Raymond et al. reported (2003) a non-toxic concentration of Aβ (200 nM) had no effect on LTD induced by 1200 pulses at 1 or 3 Hz. Similarly, Wang et al (2002) found LTD in the dentate gyrus of rat hippocampal slices was unaffected by ADDL - interestingly, their data suggested the reversal of LTD was strongly inhibited in ADDL-treated slices. Their results are in contrast to a previous report of LTD facilitation by Aβ in vivo (Kim et al., 2001). This could reflect the use of a strong LTD induction paradigm in Raymond's experiments, occluding further response depression. Alternatively, the difference may reflect the delivery method or duration of Aβ exposure. In the previous study (Kim et al., 2001), Aβ was injected intraventricularly which may have caused effects on hippocampal afferents (not present in the slice) that modulate hippocampal LTD. Furthermore, LTD was induced 10 min after intraventricular injection compared with after a 20-min bath application in Raymond's study.

1.4. Aβ and MAPK

Mitogen-activated protein kinases(MAP kinase, MAPK) comprise a group of serine/threonine kinases that are activated through multiple protein kinases in response to extracellular stimuli by dual

phosphorylation at conversed threonine and tyrosine residues (Robinson and Cobb, 1997). The MAPK module is composed of at least three kinases that established a sequential activation pathway. The top kinase of the three-kinase module is a MAPK kinase kinase (MKKK), which phosphorylates the next kinase (MKK) when activated. The MKK are kinases which recognize and phosphorylate a The-X-Tyr motif in the activation loop of MAPK. The majority substrate MAPK are transcription factor, it also phosphorylate many other substrates including protein kinases, phospholipases and cytoskeleton-associated protein such as τ protein (Fig 1.2)(Kyriakis and Avruch, 2001).

Based on the sequence similarly, mechanisms of upstream regulation and, Akhondzadeh et al (1999) to a lesser extent, substrate specificity, MAPK pathways can divided into several sub-groups amongst which extracellular signal-regulated kinases(ERK) 1 and 2, c-jun N-terminal kinase (JNK)/stress-activated protein kinase(SAPK) and p38 are the best characterized(Chang and Karin, 2001).

1.4.1 ERK pathway in nervous system

ERK is activated by MEK1 and MEK2 (Crews et al., 1992). MEKs are regulated by phosphorylation on serine and threonine residues. (Hanks and Hunter, 1995). The kinases involved in MEK1/2 activation are Raf-

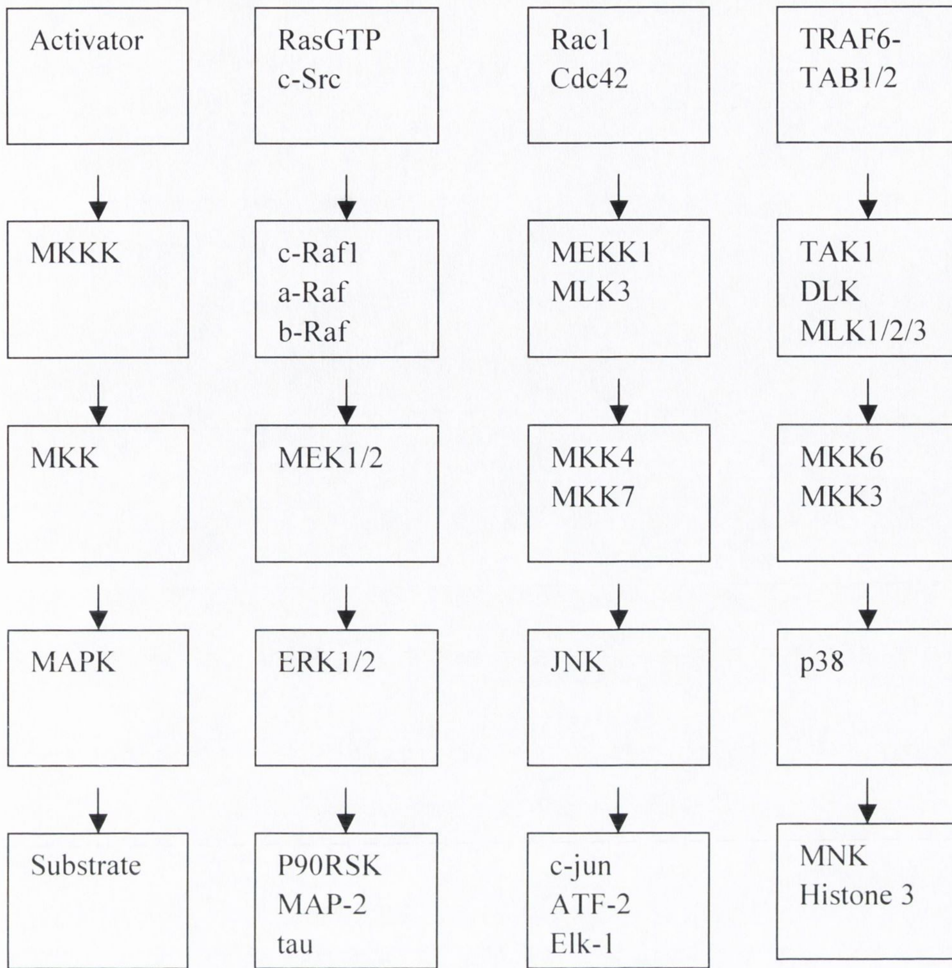

Fig 1.2 Schematic diagrams of the assembly of MAP kinase pathway.

proteins proto-oncoprotein c-Raf, A-Raf, and B-Raf) which are phosphorylated by various kinases, including at least Ras. GTP and c-Src protein tyrosine kinase (PTK), and possibly protein kinase C (PKC) and 14-3-3 proteins as well (Sugden and Clerk, 1997).

Many neurotrophins and growth factors, including NGF, BDNF, NT-3, NT-4, NT-5, bFGF and EGF, modulate the activation of ERKs in neurons, astrocytes and oligodendrocytes (Derkinderen et al., 1999). Membrane depolarization mediated by calcium influx through L-type voltage-sensitive calcium channels also activates the ERK cascade in cortical and hippocampal neurons (Hoshi et al. 1988; Ahn and Krebs 1990; Miyasaka et al. 1990). ERK activation is also regulated by a wide variety of neurotransmitter receptors coupled to either PKA or PKC, including NMDA receptors, AMPA receptors, adrenergic receptors, DA receptors, muscarinic acetylcholine receptors and metabotropic glutamate receptors (Roberson et al., 1999; Winder et al. 1999; Perkinton et al. 1999). These findings indicate an unexpected richness of diversity in ERK regulation in the hippocampus, and suggest the possibility of a broad role for the ERK cascade in both short-term and long-term forms of hippocampal synaptic plasticity. The fact that ERK activation plays a critical role in LTP induction has become clear. Accumulating data have identified a prominent role for ERK in LTP. LTP-inducing stimuli specifically increase the expression of ERK2 and B-Raf in the

hippocampus, and ERKs are essential for hippocampus and dentate LTP induction (English and Sweatt, 1997; Rosenblum et al., 2002). The role of ERK in dentate gyrus LTP and LTD in cerebellar Purkinjie neurons and hippocampus have also been reported, which may reflect a requirement for ERK-dependent, CREB- or Elk-mediated gene expression. Behavioral research in rodents provide more compelling and direct evidence for an ERK-dependent role in learning and memory (Berman et al., 1998; blum et al., 1999) since behavioral performance is associated with increased ERK activity and inhibition of ERK signaling specifically impairs learning. Initial studies in this area focused on NMDA receptor-dependent LTP in area CA1, using hippocampal slices in vitro (English and Sweatt 1996, 1997; Atkins et al. 1998; Impey et al. 1998; Winder et al. 1999; Wu et al. 1999). In addition, recent data have shown a necessity for ERK activation in the induction of NMDA receptor independent LTP (Coogan et al. 1999), LTP in the dentate gyrus in vitro (Coogan et al. 1999), LTP in vivo (McGahon et al. 1999; Davis et al. 2000; Rosenblum et al. 2000) and LTP in the amygdale (Jones et al. 1999). Strong evidence exists that ERK activation is necessary for L-LTP; three structurally distinct MEK inhibitors all block late LTP (English and Sweatt 1996, 1997; Impey et al. 1998; Atkins et al. 1998; Wu et al. 1999).

The downstream targets of activated p44/42 include cytoskeletal, nuclear and signaling proteins. For example, MAP-2, tau, neurofilaments, synapsin-1, phospholipase A2, stress-activated protein kinase (MSK), MAPK interacting kinases (MNKs) (Adams and Sweatt, 2002; Hsiao et al., 1994; Fukunaga and Hunter, 1997; Deak et al., 1998; Frodin and Gammeltoft, 1999; Smith et al., 1999; Waskiewicz et al., 1997, 1999; Kyriakis and Avruch, 2001).

The activation of ERKs can also lead to the activation of several transcription factors, including cAMP response element binding protein (CREB), Elk-1, and c-Myc. Elk1 leads to activation of serum response element on *fos* promoter, and thus c-*fos* induction (Marais et al., 1993; Whitmarsh et al., 1995; Kyriakis and Avruch, 2001). CREB activates transcription of genes when phosphorylated at Ser133; the ERK cascade is most likely coupled to CREB phosphorylation via activation of a member of the pp90rsk family of S6 kinases, RSK2. Phosphorylation of CREB recruits the CREB binding protein, CBP, to the initiator complex and thereby promotes transcription. Many genes are activated by CREB, including other transcription factors such as c-fos through which CREB signaling can indirectly activate an expanded range of genes.

At the cellular level, ERKs regulate a diverse array of functions including cell growth and proliferation, differentiation and survival or apoptosis. The most explored function of ERK is regulation of

proliferation gene expression. Gene expression is necessary for short term events into long term changes in synaptic strength.

1.4.2 JNK pathway in nervous system

JNK is a 46/54 kDa kinase, characterised by its ability to activate the transcription factor c-Jun, a component of the transcriptional activator protein, AP-1 (Kyriakis et al., 1994). JNKs are encoded by three different gene jnk1, jnk2 and jnk3. The jnk1 and jnk2 gene are ubiquitously expressed while jnk3 is selectively expressed in the brain, heart and testis. Due to alternative splicing, there are ten isoforms which differ in their interaction and catalytic alternations of the substrates (Mielke and Herdegen, 2000). JNK contains the sequence Thr-Pro-Tyr in the activation loop and the activation requires the phosphorylation of threonine and tyrosine. JKK1/MKK4 and JKK2/MKK7 are the specific physiological activator of JNK (Yang et al., 1997). Further upstream from the MKKs lie small G proteins such as Cdc42, Rac1 and MKKKs such as MLK3 and MEKK1(Coso et al., 1995).

JNKs phosphorylate a variety of nuclear proteins and regulate gene expression. Its substrates include τ protein and transcription factors such as c-Jun, ATF-2 and Elk-1(Mielke and Herdegen, 2000; Reynolds et al., 1997). Little is known about the function and activation of JNK under

physiological conditions, nonetheless, JNK appear to be involved in neuronal regeneration and neuroplasticity (Brechet et al., 1999) and a neuroprotective role for JNK is suggested in motor neurons (Migheli et al., 1997)

The activation of JNK is linked to apoptosis, and there is a numerous evidence in support of a role for JNK pathway in regulation cell death in neurons. The apoptosis of JNK in the brain were first demonstrated in JNK3 knockout mice whose hippocampus neurons were protect against excitotoxic death (Xia et al., 1995). JNK activation is implicated in the processes leading to neuronal death following nerve fiber lesions, ischemia, and seizure (Harper et al., 2001). Damage of dopaminergic neurons of substrantia nigra compacta by axon transaction in the adult rat brain results in strong activation of JNKs and consequent c-jun phosphorylation; these events precede and parallel the nigral cell death (Oo et al., 1999). JNK1 is activated in the cortex and hippocampus of rats that have been treated with kanic acid to induce seizure (Mielke et al., 1999), CEP-1347, a specific inhibitor of JNK pathway, is neuroprotectective in a number of in vivo model (DiCamillo et al., 1998). Therefore, taken together, these studies show that JNK is a potent effector of apoptosis and degeneration of neurons.

1.4.3 p38 pathway in nervous system

p38, another important MAPK that respond to cellular and environment stress, is also termed as SAPK2. There are 4 isoforms of p38 that have been identified: p38α, p38β, p38γ and p38δ, which derived from different gene (Harper at al., 2001). The p38α and p38β are widely expressed and have very high levels of expression in the brain (Jiang et al., 1996). The p38γ is expressed exclusively in skeletal muscle (Li et al., 1996), whereas p38δ is found only in lung and kidney (Jiang et al., 1997).

The p38 family is activated by dual phosphorylation on threonine 180 and tyrosine 182. The phosphorylation is mediated by the dual-specificity kinases MKK3 and MKK6 (Derijard et al., 1995). The upstream pathways of MKK are further diversified, which account for various stimuli. Several MKKKs have been reported to cause p38 activation including MLK1, MLK2, MLK3 DLK, ASK1 AND TAK1(Harper et al., 2001). The p38 pathway can be regulated by a variety of extracellular stimuli including growth factor such as GM-CSF, FGF, IGF, VEGF and PDGF, heat shock, cell stretching, proinflammatory cytokines and oxidative stress, which can lead to kinds of cellular Reponses including cell growth, cell cycle, cell death, cell differentiation and inflammation (Harper et al., 2001). The action of p 38 seems to be cell type- and stimuli-specific.

The relatively high basal level of p38 activity in neurons suggests that p38 may play an important physiological roles such as in LTP and LTD (Coogan et al., 1999). Another critical role of p38 involves in neurodegeneration (Harper et al., 2001). Inhibition of p38 MAPK has been shown to inhibit LTP (Coogan et al., 1999), and be neuroprective following permanent focal stroke in rat (Barone et al., 2000).

A novel function of p38 MAPK is phosphorylation and phosphoacetylation of histone H3 in response to inflammatory stimuli (Saccani et al., 2002). The p38 MAPK-dependent H3 phosphorylation modifies promoter regions of specific genes, such as interleukin-8 (IL-8) and monocyte chemoattractant protein 1 (MCP 1), resulting in opening of NF-αB binding sites and thereby promoting transcriptional activation. p38 MAPK activation through various pathways has been demonstrated to be essential for IL-1, IL-6, TNF-α, cyclooxygenase-2 (COX-2), and inducible nitric oxide synthase (iNOS) expression (Lee et al., 1994; Da Silva et al., 1997; Ridley et al., 1997; Bhat et al., 1998; Guan et al., 1998; Miyazawa et al., 2003). In some cell lines, the p38 MAPK pathway increases COX-2 and TNF-α expression by increasing mRNA stability and protein translation, but the mechanism of these actions are still unclear (Ridley et al., 1998; Lee et al., 2000).

1.4.4 MAPK in Aβ neurotoxicity

Both activation of p38, JNK MAPK and p44/42 MAPK pathways occur in Alzheimer's disease. Immunohistochemical analysis of postmortem AD brains revealed increased levels of activated p38 MAPK compared with age-matched control brains (Hensley et al., 1999). p38 MAPK activity was associated with the hallmark lesions of AD, the neuritic plaques, neurofibrillary tangle-bearing neurons, and neuropil threads. As phosphorylation of p38 MAPK is a response to cellular stress, it is not likely that p38 MAPK activation is an initiating step in the disease cascade. However, activation of the p38 MAPK pathway in neurons and glial cells may contribute to the degeneration or further activation of these cells. Transgenic mice overexpressing human APP develop early AD-like changes, including diffuse, extracellular Aβ deposits in brain parenchyma, as well as specific spatial learning and memory deficit (Quon et al., 1991; Higgins et al., 1994, 1995; Koistinaho et al., 2001). Interestingly, these mice showed approximately threefold elevation in the number of activated p38 MAPK-positive cells in the brain (Koistinaho et al., 2002).

Finding of increased p38 MAPK activation is seen in a mouse model of AD with more advanced amyloid pathology (Lappalainen et al., 2002). Altogether, the observation that p38 MAPK is activated in microglia of APP transgenic mice suggests that plaque-associated Aβ is not necessary

for MAPK activation in microglia, and that secreted forms APP or soluble Aβ may be sufficient. Numerous in vitro studies have demonstrated that while fibrillar Aβ peptides induce rapid activation of both p38 and p44/42 MAPKs, resulting in increased TNF-a and NO release (McDonald et al., 1998; Pyo et al., 1998; Combs et al., 1999, 2001), fresh Aβ peptides activate p44/42 MAPK in microglia only when CD45 receptor is inactivated with tyrosine phosphatase inhibitors (Tan et al., 2000). It should be noted that in the AD brain a complex inflammatory process, involving also neurons and astrocytes, is activated. It is possible that the interaction of activated astrocytes and microglia together with the neurons stressed by Aβ peptides and neurofibrillary tangles sensitize microglia to extracellular stimuli, which then trigger MAPK pathway and cytokine release.

There are some contradictory results concerning the effects of Aβ on ERK pathway. Whether Aβ leads to ERK activation or ERK activation contributes to Aβ cytotoxicity are both under debate. Major four results are: (1) Aβ induces a sustained ERK activation and ERK activation contributes to Aβ-mediated τ phosphorylation and neurodegeneration (Rapoport and Ferreira, 2000; Kuperstein and Yavin, 2002); (2) Aβ does not activate ERK pathway, but ERK mediates multiple aspect of Aβ-induced neurotoxicity (Wei et al., 2002); (3) Aβ activates ERK pathway, but ERK activation has no effect or even some protective effect on Aβ-

induced neurotoxicity (Ekinci et al., 1999). (4) Both of them have no activation each other at all (Abe and Saito, 2000). It is also under debate whether Aβ has an effect on the downstream target of ERK pathway, CREB. One group reported that Aβ impairs CREB phosphorylation (Tong et al., 2001) while another one suggested that CREB phosphorylation is increased by Aβ (Sato et al., 1997). The contradictions are likely due to the the relatively high basal level activity of ERK pathway in neuronal cultures and the relatively small effect that Aβ have on the ERK pathway, specially in the presence of some additional factors.

Studies from several groups consistently show that Aβ induced a 2-3 fold activation of JNK in different neuronal cell types and that this activation directly contributes to Aβ -induced cell death (Wei et al., 2002; Troy et al., 2001; Morishima et al., 2001). In the latter, the toxic effects of Aβ are suppressed by the specific JNK inhibitor, CEP-1347 (Troy et al., 2001), or the expression of dominant negative upstream activator, SEK1 (Wei et al., 2002 and Morishima et al., 2001). In fact, the upstream activator MEKK1 and downstream effector c-jun show high inducibility in response to Aβ and c-jun also plays an important role in Aβ cytotoxicity. Interestingly, JNK activation is localized to the Aβ deposit within neurites containing phosphorylated τ in mice double transgenic for β APP and PS. This not only suggests that JNK is activated by Aβ in neurons but also suggests that activation of JNK contribute to Aβ-induced

hyperphosphorylation of τ in vivo. By intracerebroventricular injection of A β 1-40 in the hippocampus, Minogue et al. (2003) reported that A β 1-40 induced activation of JNK in CA1 and that this was coupled with expression of the pro-apoptotic protein, Bax, cytosolic cytochrome c, poly-(ADP-ribose) polymerase cleavage, and Fas ligand expression in the hippocampus. Their data indicate that Aβ1-40 inhibited expression of long term potentiation, and this effect was abrogated by administration of the JNK inhibitor peptide, D-JNKI1. Similarly, Derek et al (2004) also found that SP600125, a JNK specific JNK inhibitor attenuated the Aβ25-35 mediated impairment of PTP and LTP

1.5 Role of NMDARs in the effect of Aβ on plasticity

There are some contradictory results concerning the effects of Aβ on the NMDA receptor. Wu reported that Aβ 1–40 (200 nM) can selectively elicit a rapid and persistent increase in NMDA-EPSC, but not AMPA-EPSC in the dentate gyrus in vitro (Wu et al. 1995). Moreover, the delayed reduction in baseline synaptic transmission in the CA1 area in vivo caused by Aβ 1–40 (3.5 nmol) can be prevented by treatment with the NMDA receptor antagonist CPP (7 mg kg21 x2, i.p.) (Cullen et al. 1996). Transgenic mice overexpressing hAPP (V717F) had a relative upregulation of NMDA receptor-mediated synaptic transmission at a time

when AMPA receptor-mediated transmission was reduced (Hsia et al. 1999). Consistent with an age-related increased potential for NMDA receptor-dependent excitotoxicity, Fitzjohn et al. (2001) reported that the non-selective glutamate receptor antagonist kynurenic acid (1 mM), when present at the anoxic period of slice preparation, prevented the reduction in baseline transmission at 12 months in hAPP K670N/M671L mice. However, this strategy was not effective at a later age (18 months) or at 8–9 months in V717F mice(Hsia et al. 1999; Chapman et al. 2001). In this context, it is interesting that glutamate can potentiate the inhibitory effect of Aβ 1–42 on LTP (Nakagami and Oda 2002). In addition, MK801, an NMDA receptor antagonist, inhibited Aβ cytotoxicity when administrated simultaneously with Aβ (Shimohama and Kihara, 2001) , the results suggest that Aβ cytotoxicity is mediated via the NMDA receptor or via glutamate in cultured cortical neurons, although Aβ can kill many types of cells without NMDA receptors (Gridley et al 1997; McLaurin et al 1999).

By contrast, Raymond CR et al. (2003) reported that Aβ can cause a rapid, albeit modest, down-regulation of NMDAR function. Aβ also inhibited LTP in an NMDAR-independent manner. These authors suggested that endogenous Aβ may serve a normal regulatory function in opposing both NMDAR activation and LTP induction by different mechanisms. In other studies, Aβ 1–42 (200 nM and 1 mM) has been

reported to reduce NMDA receptor-mediated synaptic currents in the dentate gyrus (Chen et al., 2002). Aβ 1–42 and ADDLs at the sublethal concentrations of 5 mM and 100 nM, respectively, also strongly suppressed a NMDA-evoked/depolarization-induced increase in CREB phosphorylation in cultured cortical neurons, whereas Aβ 25–35 (10 mM) was inactive (Tong et al., 2001). CREB phosphorylation has been implicated in late LTP. In a recent study, rolipram and forskolin, agents that enhance the cAMP-signaling pathway, reversed the inhibition of LTP by Aβ 1–42. This reversal was blocked by H89, an inhibitor of protein kinase A (Vitolo et al., 2002).

An intriguing corollary to the block of LTP by Aβ is the facilitation of LTD induction by low-frequency stimulation and time-dependent LTP reversal in the CA1 area by very low-dose Aβ 1–42 (1 pmol i.c.v.) and CT105 (1–2 pmol), respectively. Both effects were blocked by the NMDA receptor antagonist D-AP5 (100 μmol), indicating their NMDA receptor dependence (Kim et al., 2001). Somewhat similarly, Aβ 1–42 applied in the first hour after HFS inhibited LTP, and inhibition of calcineurin activity with FK506 or cyclosporin A completely prevented this effect (Chen et al., 2002). By contrast, ADDLs (500 nM) failed to affect a large, apparently NMDA receptor-independent form of LTD in the dentate gyrus of young (14–19-day-old) rats (Wang et al., 2002).

1.6 Aβ and oxidative stress

Several different studies have provided evidence implicating oxidative stress as a major pathogenic mechanism in AD (Mark et al., 1996; Good et al 1996). The concept of oxidative stress refers to a state in which oxidant production surpasses the endogenous antioxidant capabilities leading to oxidative molecular damage of the tissue. Such a state can be achieved either by increased production of cellular oxidants and/or decreased concentrations of cell antioxidants including glutathione, vitamin E, ascorbate, the glutathione peroxidase system, superoxide dismutase (SOD), and catalase. Overproduction of reactive oxygen and nitrogen species such as superoxide, hydrogen peroxide, nitric oxide, and peroxynitrite occurs in AD and is considered to mediate cellular damage and signal apoptosis.

Cultured rat and human hippocampal and cortical neurons are vulnerable to self-aggregated Aβ fibrils and such toxicity may be prevented by antioxidants. Aβ induces the formation of unusually high concentrations of oxygen and nitrogen-reactive species and a depletion of endogenous antioxidants that play a central role in damaging and killing neurons (Mark , et al., 1996). In brain tissue from AD patients, there are increased levels of markers of oxidative stress including oxidized proteins, membrane lipids, DNA, and nitrotyrosine immunoreactivity (Smith, et al., 1998). Additionally, antioxidants including vitamin E,

idebenone, uric acid, and glutathione are effective in preventing Aβ neurotoxicity *in vitro* . Some of these compounds also showed beneficial effects in patients with AD, slowing the progression of the disease (Bruno , et al., 1994; Mayeux and Sano , 1999).

The biochemical pathways by which Aβ initiates direct neurotoxicity in neurons involve the Aβ -dependent generation of reactive oxygen species (Mattson , 1997), including the generation of hydrogen peroxide, peroxyl radical, and superoxide that in turn may initiate a series of secondary reactions between radicals and other biomolecules. It is worth mentioning that it has been reported that free radicals induce massive calcium entry into neuronal cells (MacManus 2000,Atwood et al., 1988).hydrogen peroxide will produce the strong oxidant and cytotoxin hydroxyl radical. Recent studies have shown that free radicals can also be formed following activation of the receptor for advanced glycation end products or type 2-scavenger receptors, which respond to Aβ binding by stimulating the activity of NADH oxidases (Yan et al., 1996). Although nitridergic species such as nitric oxide and peroxynitrite are overproduced and may contribute to the pathogenesis of AD, it is likely that their production is dependent on the activation of secondary inflammatory mechanisms involving activated glial cells, which may represent an important source of NO (Hu et al 1998). Another relevant aspect to be considered is the involvement of oxidative stress in brain

cells derived from iron metabolism, which may be responsible for altering transduction mechanisms in neuronal cells as related to neurodegeneration (Smith, et al., 1997).

1.7 Microglia and Aβ

Several groups have reported that activated microglia and pro-inflammatory molecules are present at the sites of extracellular lesions (McGeer and McGeer, 1999; Akiyama et al., 2000; Halliday et al., 2000). Further evidence for the involvement of inflammation in the pathogenesis of AD is offered by epidemiological findings showing delayed onset and slowed progression of AD among long-term nonsteroidal anti-inflammatory drug (NSAID) users (McGeer et al., 1996).

The presence of activated microglial cells represents the major and most characteristic feature of inflammation in AD brain. Fibrillar and other components of the neuritic plaques can activate microglia, which in turn have the potential ability to remove Aβ. Although microglia cells constitute approximately 10–20% of the glia population, their numbers can rapidly increase. Activated microglia produce chemotactic factors that further maintain microglial activation and a number of complement proteins that exert direct cytotoxicity. Aβ can bind and activate C1q complement factor and via this mechanism can activate the full cascade

of the complement pathway (Jiang et al., 1994). In addition, formation of the complement-dependent membrane attack complex may exert local toxicity in neurites, explaining the characteristic synapse loss in AD (McGeer et al., 1995). It is recognized that there is an increased expression of complement factors and markers of complement activations in AD (Yasonima et al., 1999). Microglia and astrocytes also produce a variety of cytokines and chemokines, including interleukin 1b, interleukin 6, and tumor necrosis factor-alpha, known to be associated with immunologic and inflammatory reactions and found up-regulated in AD-affected brains.

Activated microglia produce large amounts of NO and superoxide following expression of iNOS and activation of NADPH oxidase. In addition, microglia represent a potential source of low-molecular-weight excitotoxins that may further aggravate exocytotoxicity (Liberatore et al., 1999). The phenomenon by which microglia exert neurotoxicity was described as autotoxicity by McGeer and McGeer (McGeer et al., 1995). Nitric oxide is a free radical with low reactivity and toxicity that in itself is particularly adapted to serve as an intra- and inter-cellular messenger in many biological systems. Nitric oxide and superoxide can combine by a diffusion-limited reaction to form the much stronger and more toxic oxidant peroxynitrite, which probably accounts for much of NO cytotoxicity *in vivo* . Peroxynitrite reacts with the majority of the

components in the cells, including thiols, thiol ethers, iron sulfur centers, and zinc fingers and initiates lipid peroxidation. In addition, it combines with free and protein-bound tyrosine to form nitrotyrosine (Beckman et al., 1996), a stable product that can be used as an indicator of peroxynitrite production. Evidence for peroxynitrite production in AD is obtained from reports describing increased levels of nitrotyrosine in the brain tissue of AD patients (Su et al. 1997; Smith et al., 1997 and Hensley et al.,1998).

In AD-affected brains, activated microglia surround Aβ plaques. These activated glia contribute to neurotoxicity through the induction of inflammatory mediators such as IL-1 and tumor necrosis factor-alpha (TNF-α). In addition, these cytokines mediate the expression of the inflammatory enzyme-inducible nitric-oxide synthase (iNOS) (Akama, et al 2000). Exposure of rat cortical astrocyte cultures to Aβ induced activation, as assessed by reactive morphologic changes and up-regulation of interleukin-1β; and iNOS expression (Hu et al., 1998), suggesting that Aβ plays a role in the reactive glia.

Astrocytes represent the largest cell population in the CNS. They closely interact with neurons across the extracellular space to provide structural, metabolic, and trophic support and actively participate in modulating neuronal excitability and neurotransmission by controlling the extracellular levels of ions and neurotransmitters. Under normal

conditions, astrocytes are particularly adapted to respond to mild oxidative stress and to protect neurons from reactive oxygen species (ROS). However, oxidative stress may directly activate specific transcription factors, resulting in an up-regulation of inflammatory mediators and enzymes including inducible nitric oxide synthase .

Gap junctions between astrocytes are impaired after oxidative damage (Saez , et al., 1990), preventing calcium waves or buffering of high levels of ions and thus indirectly affecting neuronal excitability or neurotransmission. Peroxynitrite and the induction of iNOS in astrocyte astrocyte monolayers inhibit gap junction permeability (Bolaños et al., 1996), thus preventing astrocytes from functioning properly in the homeostasis of ions and neurotransmitters and promoting neuronal toxicity. Aβ -induced oxidative stress in astrocytes may contribute to disrupting the function of glutamate transporters in AD (keller et al., 1997). Excitatory amino acid transmission is dependent upon rapid clearance of released glutamate from the extracellular space by high affinity glutamate transporters located in the plasma membrane of presynaptic terminals and astrocytes. Blockage of glutamate uptake results in receptor overstimulation with subsequent increased neuronal activity that may lead to neuronal death (Lipton et al., 1994). Evidence that ROS inhibit high affinity glutamate uptake in astrocytes and synaptosomes was provided by Volterra and co-workers (Volterra et al.,

1994). This seems to be due to direct oxidation of critical sulfhydryl groups in the transporter protein. Such damage may be reproduced by peroxynitrite or hydrogen peroxide. Importantly, glutamate uptake was also inhibited by Aβ peptide in rat hippocampal astrocyte cultures (Parpura-Gill, et al., 1997), an effect prevented by the antioxidant trolox, suggesting that lipid peroxidation may also play a role in this model (Harris et al., 1998).

Reactive-astroglial phenotypes induced by Aβ may contribute to neuronal damage and disease progression in AD by producing neurotoxic concentrations of nitric oxide. Aβ provokes reactive morphologic changes in astrocytes and up-regulation of cytokines and iNOS through an NFkappaB-dependent mechanism (Hu et al 1998 and Akama et al., 1998). Astrocyte-mediated neurotoxicity has been associated with astrocytic iNOS expression in several *in vitro* and *in vivo* models. In addition, induction of astrocytic NOS in mixed cultures of cortical neurons and astrocytes potentiates NMDA-induced neuronal injury (Hewett SJ, et al 1994) or induces direct neuronal toxicity by a NO-dependent mechanism (Dawson et al., 1994 and Chao et al., 1995)

1.8 Acetylcholine receptor and Aβ

Several lines of evidence indicate that Aβ can inhibit various steps in the synthesis and release of ACh, thus suggesting a link between amyloid

burden and cholinergic impairment in AD (Kar et al., 1996, 1998; Hoshi et al., 1997; Wang et al., 1999; Lee et al., 2001; Krištofiková et al., 2001; Vaucher et al., 2001), and these effects are independent of any apparent neurotoxicity. The first evidence came using rat hippocampal and cortical slice preparations treated with various fragments of Aβ (Kar et al., 1996). Kar and his colleague found that ACh release from hippocampal and cortical slices stimulated with high-K+ or veratridine was rapidly inhibited by pM to nM concentrations of Aβ. This effect was not modified by tetrodotoxin, implying that Aβ acts at the level of the cholinergic nerve terminal (Kar et al., 1996). Aβ1–42,

Aβ1–40, Aβ25–35 and Aβ1–28 all reduce ACh release, thus implicating the sequence Aβ25–28 as being crucial for the effect. However, ACh release from striatal slice preparations was not sensitive to reduction by this peptide (Kar et al., 1996). Vaucher et al (2001) demonstrated that hippocampal Ach release from cognitively impaired aged rats which have higher levels of hippocampal Aβ is more sensitive to Aβ1–40 than is ACh release from cognitively unimpaired animals . As opposed to direct Aβ induction of cognitive dysfunction, these results imply that a priori cognitive status can predict the sensitivity of the cholinergic system to exogenously applied Aβ peptides (Vaucher et al., 2001). These data also suggest that the particularly high sensitivity of

ACh release to endogenous Aβ might contribute to naturally occurring cognitive impairments.

Aβ1–42 has also been shown to reduce ACh release from cortical synaptosomes (Wang et al., 1999). By using a pure population of cholinergic synaptosomes, Satoh et al (2001) clearly indicate that Aβ acts on cholinergic terminals, rather than acting indirectly via another neuronal phenotype or glial cell. Reductions in ACh concentration, in the absence of concurrent neurotoxicity, have also been reported in primary septal cultures following a 12-h treatment with nM concentrations of solubilized Aβ1–42 (Hoshi et al., 1997). Aβ exposure of these primary neurons did not reduce ChAT activity, but the activity of pyruvate dehydrogenase (PDH) was reduced (Hoshi et al., 1997).

PDH is only in cholinergic neurons that it is also important for ACh synthesis. Thus, neurotransmission in these neurons may be selectively impaired by the inhibition of PDH by Aβ. Interestingly, reduced PDH activity has been observed in AD (Sorbi et al., 1983), stressing the potential importance of these findings.

In a hybrid cell line created from the fusion of mouse septal neurons and neuroblastoma cells (SN56), intracellular ACh concentration was reduced after a 48-h treatment with nM concentrations of Aβ25–35, Aβ1–28 or Aβ1–42 without indications of toxicity (Pedersen et al., 1996; Pedersen and Blusztajn, 1997). A reduction in ChAT activity was

associated with the reduction in ACh concentrations that accompanied prolonged Aβ treatment (Pedersen et al., 1996). By contrast, acetylcholinesterase (AChE) activity was not reduced following Aβ treatment.

Aβ impairment of hippocampal LTP possibly involve nicotinic receptors (Cullen et al., 1997; Itoh et al., 1999; Ye and Qiao, 1999; Chen et al., 2000). For example, the inhibition was prevented by huperzine, an AChE antagonist expected to increase ACh concentrations in the synaptic cleft (Ye and Qiao, 1999).

Other evidence suggests that Aβ disruption of LTP may limit the normal supply of NGF, and thus contribute to AD. Considering that retrograde transport of NGF is likely impaired in AD, resulting in normal or increase NGF levels in target areas , potential dysregulation of local neuromodulatory functions of NGF (Knipper et al., 1994; Sala et al., 1998; Auld et al., 2001) may be most sensitive to this disruption.

Treatment with either Aβ25–35 or Aβ1–40 (1μM) has been shown to decrease whole cell voltage-sensitive currents in cholinergic neurons identified by single cell RT-PCR (Jhamandas et al., 2001). This reduction is associated with changes in several K^+ currents, including the Ca^{2+}-activated K^+ current, the delayed rectifier current and transient outward K+ conductances. In contrast, Aβ did not modulate either Ca^{2+} or Na^+ currents. Aβ modulation of K^+ currents at the level of the cholinergic cell

body increases excitability, resulting in a higher frequency of action potential generation (Jhamandas et al., 2001).

A 4-h exposure to Aβ 25–35 (nM to μM concentrations) reduces carbachol stimulated GTPase activity in primary cultures of rat cortical neurons (Kelly et al., 1996). At higher concentrations, similar treatment with Aβ reduced the accumulation of intracellular messengers associated with M1-like receptor signaling, including reduced impaired Ca2+ signaling and inositol second-messenger accumulation (i.e. Ins(1)P, Ins(1,4)P2, Ins(1,4,5)P3 and Ins(1,3,4,5)P4 (Kelly et al., 1996). Aβ effected these signaling alterations without modifying receptor-binding kinetics. Interestingly, the anti-oxidants Vitamin E and propyl gallate prevented the effect of high concentrations of Aβ on GTPase activity (Kelly et al., 1996). Moreover, it was shown that a 24-h exposure to Aβ1–42 and Aβ 25–35 (100 nM) inhibited increases in intracellular Ca2+ and Ins(1,4,5)P3 induced by muscarine (Huang et al., 2000). this effect was selective for muscarinic receptors and was attenuated by the anti-oxidant Vitamin E (Huang et al., 2000). These studies indicate that disruption of muscarinic signaling, perhaps involving the generation of reactive oxygen species.

In AD, M1-like receptor levels are equivalent to those of age-matched controls (Araujo et al., 1988; Quirion, 1993). However, most studies report robust reductions in G-protein signaling (Smith et al., 1987;

Ferrari-DiLeo and Flynn, 1993; Warpman et al., 1993; Ferrari-DiLeo et al., 1995) and/or phosphoinositide generation (Jope et al., 1994, 1997; Greenwood et al., 1995) after muscarinic receptor stimulation in AD brain tissue. Considering the studies suggesting that Aβ disrupts muscarinic signaling in vitro (Kelly et al., 1996; Huang et al., 2000), it is possible that Aβ also makes contributions to the disruption of muscarinic signaling observed in the AD brain.

ACh also signals through nicotinic receptors (Aubert et al., 1992, 1996; Clarke, 1995). Shimohama and Kihara (2001) reported that simultaneous incubation of the cultures with nicotine and Aβ significantly reduced the Aβ-induced cytotoxicity. The protective effect of nicotine was reduced by both DHβE and α-BTX. β-amyloid cytotoxicity was significantly reduced when 10 mmol/L cytosine, a selective α4β2 nicotinic receptor agonist, or 1 mmol/L DMXB, a selective α7 nicotinic receptor agonist, was co-administered. These findings suggest that both α4β2 and α7 nicotinic receptor stimulation are protective against Aβ cytotoxicity (Kihara et al 1997, 1998). Moreover, they also found that nicotinic receptor mediated neuroprotection against β-amyloid-enhanced glutamate toxicity. The α7 nicotinic receptors have high Ca^{2+} permeability and are known to be involved in numerous processes, including neurotransmitter release (Alkondon et al., 1997), long-term

potentiation (Mansvelder and McGeher, 2000; Ji et al., 2001) and learning (Levin and Simon, 1998; Jones et al., 1999).

It has also been reported that there are alterations in nAChR expression levels in AD brain regions such as the hippocampus and cortex (Quirion, 1993; Court et al., 2000; Paterson and Nordberg, 2000; Nordberg, 2001). In terms of specific subunits, α7nAChR levels have been reported to be reduced in the hippocampus (Guan et al., 2000) but not in the cortex (Martin-Ruiz et al., 1999; Guan et al., 2000), of AD patients. In addition, expression levels of nAChRs with α4 subunits have been demonstrated to be reduced in the hippocampus (Guan et al., 2000), and reduced (Guan et al., 2000) or unchanged (Martin-Ruiz et al., 1999) in the cortex of the AD brain. Similarly, the α3 nAChR subunit has been shown to be reduced in the hippocampus (Guan et al., 2000) and either reduced (Guan et al., 2000) or unaltered in the cortex (Martin-Ruiz et al., 1999) in AD. Thus, although there are definite alterations in nAChR distribution and levels in AD, there is not yet a consensus concerning their exact nature. Given the disruption in nAChR levels in AD, it is notable that there are several different interactions between Aβ peptides and nAChRs—most notably with the α7 subtype. These interactions are believed to have relevance to AD by means of their involvement in the disruption of normal cholinergic neurotransmission and the possible

enhancement of sensitivity to Aβ neurotoxicity (Wang et al., 2000; Dineley et al., 2001; Liu et al., 2001; Pettit et al., 2001).

The first reported observation of an interaction between Aβ and α7 nicotinic receptors showed that these proteins co-immunoprecipitated in human brain tissue, particularly in the AD brain (Wang et al., 2000). Notably, Aβ1–42 and α7nAChRs were co-localized on cortical neurons and neurotic plaques. In vitro, the formation of Aβ1–42/α7 nicotinic receptor complexes was inhibited by the Aβ12–28 fragment, suggesting that this sequence competes for the binding site and is therefore likely to contain the active site (Wang et al., 2000). A subsequent study indicated a high affinity interaction between Aβ1–42 and α7nAChRs, with a Ki of ~4–5pM (Wang et al., 2000). Aβ1–42 was also shown to interact with α4β2nAChRs, albeit with lower affinity (a Ki of ~20–30 nM) (Wang et al., 2000). but several groups didn't find the capacity of Aβ to displace nicotinic ligand binding(Guan et al., 2001; Liu et al., 2001).

In hippocampal slices, solublized Aβ1–42 rapidly reversibly, inhibited carbachol-induced whole-cell nicotinic currents (Pettit et al., 2001). At the level of single nicotinic channels, Aβ reduced open probability within milliseconds. These data are consistent with a direct and specific binding of Aβ to these receptors.

It has also been reported that nM concentrations of Aβ1–42 and Aβ1–40 rapidly (within 1 min) blocked up to 80% of the whole-cell

α7nAChR current in cultured primary embryonic hippocampal neurons (Liu et al., 2001). In contrast to another study indicating that Aβ also inhibits non-α7nAChR currents (Pettit et al., 2001) and biochemical studies showing high affinity (*K*i in the nM range) interactions between Aβ1–42 and α4β2 nAChRs (Wang et al., 2000a), Liu et al. (2001) found that Aβ did not alter non-α7nAChR current.

Aβ inhibited non-α7 AChR currents in the hippocampal slice preparations taken from 13 to 18 days old rats in which interneurons were the focus (Pettit et al., 2001). By contrast, Aβ did not affect the non-α7 AChR current component in hippocampal cultures taken from embryonic days 18–19 rats, which have a prominent pyramidal cell population (Liu et al., 2001).

Aβ's reduction of the α7nAChR current was reversible and was restored to control level within 5 min of its removal (Liu et al., 2001). The impairment of this current by Aβ was not dependent on voltage, but was critically dependent upon the extracellular N-terminal length of the α7 subunit (Liu et al., 2001). The fact that Aβ did not alter glutamate- or GABA-induced currents argues in favour of a specific effect of Aβ on α7nAChR currents (Liu et al., 2001). The capacity of Aβ to reduce the α7nAChR current was not associated with competitive displacement of α-BTx binding to this receptor site. Indeed, in contrast to previous reports using membrane homogenates (Wang et al., 2000), Aβ did not alter αBTx

binding in these cultures (Liu et al., 2001). In addition, it was reported in PC12 cells that Aβ does not complete with either αBTx or epibatidine binding sites (Guan et al., 2001).

Activation of pre-synaptic nAChRs is known to stimulate neurotransmitter release from cultured hippocampal neurons (Alkondon et al., 1997). Liu et al. (2001) observed that glutamate-dependent mEPSC frequency is increased by nicotine. In keeping with the capacity of Aβ to block nAChR mediated currents, exposure to nM Aβ1–42 blocked the effect of nicotine to increase excitatory neurotransmission (Liu et al., 2001). The observations that Aβ can disrupt α7 and non-α7nAChR function, and that α7 (Gray et al., 1996) and non-α7 receptors (α4β2 nAChR; Quirion et al., 1994; Nordberg et al., 1989) can modulate hippocampal ACh release, lend support to the possibility that Aβ-inhibition of nicotine-induced neurotransmitter release could have highly significant effects on activity in circuitry innervated by BFCNs. Interestingly, Itoh et al. (1996) observed that chronic exposure to Aβ reduced nicotine-induced ACh release from the hippocampus, but that K+-stimulated ACh release was not affected. This raises the intriguing possibility that an interaction between Aβ and nAChR may contribute to this effect.

In addition to direct Aβ modulation of nAChR currents, there is evidence for a complex relationship between Aβ, α7nAChRs, and MAPK

phosphorylation (Dineley et al., 2001). Nicotine induces ERK2 MAPK phosphorylation in cultured rat hippocampal slices. Aβ1–42 treatment also results in ERK2 MAPK phosphorylation within minutes (Dineley et al., 2001). When hippocampal slices were treated with Aβ in the presence of specific α7nAChR antagonists, ERK2 MAPK was not activated, suggesting that these receptors are involved in activation of ERK2 MAPK (Dineley et al., 2001). This data support that derangement of hippocampus signal transduction cascades in AD arises as a consequence of increased Aβ burden and chronic activation of the ERK MAPK cascade in an α7 nAChR-dependent manner that eventually leads to the down-regulation of ERK2 MAPK and decreased phosphorylation of CREB protein.

Undoubtedly, these differences reflect the complexity of Aβ's interactions with multiple levels of the cholinergic system, as well as the mechanisms through which nicotine induces MAPK phosphorylation.

1.9 Tau Protein and Aβ

In 1986, Brion and co-workers showed that antibodies against the cytoskeletal protein tau labeled neurofibrillary tangles; these studies were complemented by findings in several laboratories that tau is the main component of PHFs (Kosik et al., 1986). NFTs composed of arrays of PHFs are present mainly in the hippocampus, entorrhinal cortex, and

amygdala. PHFs are anomalous structures generated by self-aggregation of hyperphosphorylated forms of tau protein that form a compact filamentous network (Maccioni et al., 1995; Mandelkow et al., 1995). Tau is a multifunctional microtubule-associated protein that plays major roles in the assembly of microtubules, the stabilization of microtubules against dynamic instability, and in bridging these polymers with other cytoskeletal filaments (Kosik et al., 1986; Maccioni et al., 1995; Mandelkow et al., 1995). In normal brain, the equilibrium between phosphorylations and dephosphorylations of tau modulates the stability of the cytoskeleton and consequently axonal morphology. The earliest modification found in Alzheimer brains consists of hyperphosphorylations on tau by the action of different protein kinase and phosphatase systems that appear to lead to structural and conformational changes in this protein, thus affecting its binding with tubulin and the capacity to promote microtubule assembly (Maccioni et al., 1995; Schweers et al., 1995). Among kinases involved in tau modifications leading to PHFs, two are the most relevant: tau protein kinase I (TPK I) also named glycogen synthase kinase 3β (GSK3β)(Takashima et al., 1993; Imahori et al., 1997) and tau kinase II (TPK II) (Alvarez et al., 1999; Patrick et al., 1999). TPK II is a complex of two subunits: a catalytic component of 33 kDa (Cdk5) and the regulatory subunit of 23 kDa (p35) derived from proteolytic cleavage of a 34 kDa precursor

(Pigino et al.; 1997 Paglini et al., 1998). The change in Cdk5 regulatory patterns was concomitant with neuronal death of hippocampal neurons and tau hyperphosphorylation, pretreatment of cells with Cdk5 specific inhibitors such as butyrolactone I or by using anti-sense probes for Cdk5-protected cells against neuronal death, and tau phosphorylation. Neuroprotection of cells to the neurotoxic effects of fibrillary Aβ strongly suggests that alterations in Cdk5 regulation play a key role in the sequence of molecular events that determine neurofibrillary degeneration in hippocampal cells. Present evidence supports the idea that in the neurodegenerative pathway, the initial anomalous tau phosphorylations by Cdk5 stimulate the modifications by GSK3β , thus preventing tau from incorporating into microtubules (Alvarez et al., 1999). In AD, some authors showed that the cleavage of p35 to p25 with the subsequent release of p25 activator seems to be responsible for the activation of this protein kinase during fibrillary neurodegeneration (Patrick et al., 1999; Ahlijanian et al., 2000; Lee et al., 2000). In the context of the preceding information, it appears reasonable to think that the reactions leading to neuronal death in this disease are part of an integrated process involving extracellular and intracellular changes in the normal neuronal machinery, in which Aβ deposition appears to be one of the promoting factors . Studies showed that Aβ-induced neurotoxicity in embryonic rat hippocampal cells and a neuroblastoma line (N2A) markedly diminished

with co-incubation of neuronal cells with Aβ plus the Cdk5 inhibitor butyrolactone I (Alvarez et al., 2001). These studies indicate that the protein kinase Cdk5 plays a major role in the molecular pathway leading to the neurodegenerative process triggered by amyloid fibers in neuronal cells. Recent investigations strongly support the role of Cdk5 in neuronal differentiation (Muñoz et al., 2000), as well as in the apoptosis of human glial cells (Catania et al., 2001).

1.10 The aim of present study

Although Thousands of papers on Aβ have been published, little is known about the mechanisms involved in Aβ-mediated inhibition of LTP. The aim of the present studies was two-fold. Firstly, we wished to determine whether naturally secreted Aβ□ inhibited LTP induction in hippocampal slices, and to compare the potency of naturally secreted and synthetic Aβ. Secondly, in order to understand further the mechanisms underlying the inhibition of LTP induction by Aβ, we examined the involvement of certain kinases and transmitter receptors, namely JNK, p38 MAPK and Cdk5, and mGluRs. We also investigated the involvement of microglia, and reactive nitrogen and oxygen species, in the Aβ-mediated inhibition of LTP induction.

2 Materials and Methods

2.1 Preparation of slices

All experiments were carried out on transverse slices of the rat hippocampus (males, age 3-4 weeks, weight 40-80g) or mice aged 3-4 months. Male wild-type and i-NOS knockout C57 black mice (18-28g) were obtained from Jackson Laboratories (Bar Harbor, ME, USA). The brains were rapidly removed after decapitation and placed in cold oxygenated (95% O_2 / 5% $C0_2$) media. Slices were cut at a thickness of 350 μm using a Campden vibroslice (Lafayette, IN) and placed in a storage container containing oxygenated medium at room temperature (20 - 22°C) for 1 hr. The slices were then transferred to a recording chamber for submerged slices and continuously superfused at a rate of 5-6ml/min at 30-32°C. The control media contained: (mM) NaCl, 120; KCl 2.5, NaH_2P0_4, 1.25; NaHC03 26; MgS04, 2.0; $CaCl_2$, 2.0; D-glucose 10. For experiments on the dentate gyrus, all solutions contained 100 μM picrotoxin to block $GABA_A$-mediated activity.

2.2 Agents

Drugs used were synthetic human $A\beta_{1-42}$ (Bachem), minocycline, aminoguanidine, superoxide dismutase, DPI, catalase, D (-)-2-amino-5-phosphonopentanoic acid (D-AP5), picrotoxin, methylcaconatine (MLA) and methyl-6-(phenylethynyl)pyridine (MPEP) (all from Sigma), butyrolactone (Affiniti Research Products Ltd, UK), LY341495, 1,4-diamino-2,3-dicyano-1,4-bis(2-aminophenylthio)butadiene (UO126), NS398 (Tocris Cookson), 4-(4-fluorophenyl)-2-(4-

methylsulfonylphenyl)-5-(4-pyridyl)-1H-imidazole (SB203580) and anthrax (1,9-cd)pyrazol-6(2H)-1,9; (1,9,pyrazoloanthrone) (SP600125) (Alexis Biochemicasl), 1400W, JNKI and roscovitine (Calbiochem). LY341495, UO126, roscovitine SP600125, NS398, butyrolactone and SB203580 were dissolved in DMSO, with a maximum final concentration of 0.1% DMSO. The other agents were prepared in distilled water. Synthetic $A\beta_{1-42}$ was prepared as a stock solution of 50 mM in ammonium hydroxide (0.1%), stored at -20°C, and then added to physiological medium immediately prior to each experiment. The actual concentration of $A\beta_{1-42}$ from one batch of peptide was determined experimentally by quantitative amino acid analysis and found to be 33% lower than the nominal value.

2.3 Electrophysiological techniques

Standard electrophysiological techniques were used to record field potentials. All experiments investigating NMDAR-dependent LTP and LTD were carried out in the dentate gyrus, with presynaptic stimulation applied to the medial perforant pathway of the dentate gyrus using a bipolar insulated tungsten wire electrode, and field excitatory postsynaptic potentials (EPSPs) recorded at a control test frequency of 0.033 Hz from the middle one-third of the molecular layer of the dentate gyrus with a glass microelectrode. In each experiment, an input-output curve (afferent stimulus intensity versus EPSP amplitude) was plotted at the test frequency. For all experiments, the amplitude of the test EPSP was adjusted to one-third of maximum (~1.2 mV). LTP was evoked by high frequency stimulation (HFS) consisting of 8 trains, each of 8 stimuli at 200 Hz, intertrain interval 2s, with the stimulation voltage increased

during the HFS so as to elicit an initial EPSP of the train of double the normal test EPSP amplitude.

The experiments investigating NMDAR-independent LTP were carried out in the CA1 region, with NMDAR-independent LTP induced by 3 spaced HFS at 10 min interval, each HFS as above.

Measurements of resting potential and input resistance were carried out using whole cell patch clamp recordings from CA1 pyramidal cells using the Axopatch 1D amplifier (3 kHz low pass Bessel filter). Series resistance varied from 15-30 MΩ. Pipette solution contained: (mM) Potassium gluconate, 140; EGTA, 10; HEPES, 10; NaGTP 0.3 ; Mg_2GTP, 2.0; QX-314, 5.

In experiments involving application of Aβ, Aβ was perfused for 30-40 min prior to HFS. In experiments involving additional other agents, the agents were perfused over the slices for 60 min prior to HFS. Control (vehicle alone) and experimental levels of LTP were measured on slices prepared from the same hippocampus. In experiments involving kinase inhibitors, experiments involving the effect of kinase inhibitor alone and the effects of the kinase inhibitor applied together with Aβ were also carried out on slices from the same hippocampus.

Recordings were analysed using p-CLAMP (Axon Instruments, CA, USA). Values are the means ± S.E.M. for n slices. Two-tailed Student's t-test and two-way ANOVA with repeated measures were used for statistical comparison.

2.4 Cell-derived Aβ

Naturally secreted cell-derived human Aβ was obtained from cultures of Chinese hamster ovary (CHO) cells stably expressing human APP751, containing the Val717Phe familial AD mutation called 7PA2 cells. CHO and 7PA2 cells were cultured in DMEM with 10% fetal bovine serum, as described previously (Walsh et al., 2000). When confluent, the cells were washed with plain DMEM and then incubated in plain DMEM (4 ml/10 cm^2 dish) for 16 hr. At the end of this period, media were harvested and cleared of cells by centrifugation at 500 x g for 10 min. Aliquots of the media were then removed, and the presence of both monomeric and oligomeric Aβ was assessed by immunoprecipitation–Western blotting (ip–wb) and ELISAs. ELISAs were performed as described previously (Walsh et al., 2000). Thus, ELISAs for Aβ 1-total (all Aβ species beginning at Asp1) and $A\beta_{1-42}$ were performed using 3D6 (which recognizes the extreme N terminus of Aβ) as the capture antibody and 6C6 (which binds to the mid-region of Aβ) for detection. In detail, nearly confluent (95–100%) 10 cm^2 dishes of 7PA2 cells and their corresponding untransfected parental CHO cell line were starved of methionine for 30 min and labeled with 750 μCi of $[^{35}S]$methionine; their media were then harvested and immunoprecipitated. After electrophoresis on 16% tricine gels, bands were visualized by gel fluorography. For experiments examining the ability of Aβ oligomers to form in conditioned medium (CM) in the absence of cells, 7PA2 cells were pulsed with 1 mCi of $[^{35}S]$methionine for 2 hr. The labeled medium was harvested, cleared of cells, incubated at either 4 or 37°C for 15 hr in the presence or absence of CHO cells and then immunoprecipitated with

the polyclonal antibody R1282. As positive controls, 7PA2 cells were labeled for 17 hr, and their CM was immunoprecipitated as described above. To visualize steady-state levels of Aβ in human CSF and in cultures that were not radiolabeled, we devised an ip–wb protocol that allowed the highly sensitive detection of unlabeled Aβ species. Analysis of CM by ELISA and ip–wb revealed that our ip–wb protocol can readily detect as little as 200 pg of endogenously secreted Aβ. Samples were immunoprecipitated to avoid reconstitution procedures that might alter the assembly form or recovery of Aβ. After immunoprecipitation, samples were electrophoresed on 16% tricine gels and transferred onto 0.2 μM nitrocellulose membranes at 400 mA for 2 hr. Filters were boiled for 10 min in PBS and blocked overnight at 4°C with 5% fat-free milk in 20 mM Tris-HCl, pH 7.4, containing 150 mM NaCl and 0.05% Tween 20 (TBS-T). After washing the membranes in TBS-T, monoclonal antibody 6E10 or a combination of monoclonals 4G8 and 6C6 (each at 1 μg/ml) was used to probe the blots. Bound antibody was visualized using horse-radish peroxidase-conjugated anti-mouse Ig (at 1:40,000) (Jackson ImmunoResearch, West Grove, PA) and ECL Plus detection (Amersham Biosciences, Arlington Heights, IL).

3 Results

3.1 effect of Aβ on membrane potential, input resistance

Perfusion of Aβ (500 nM) did not alter the membrane potential or input resistance measured under whole cell current clamp conditions. The mean resting potential and input resistance was –71±8 mV and 239±14 MΩ, in control and –69±4 and 234±8 following perfusion of Aβ for 60 min ($p>0.05$, $n=4$). Moreover, Aβ (500 nM) had no effect on the base line input-output curve(stimulation voltage versus field EPSP) ($P>0.05$, Fig3.1).

3.2 Effect of Aβ on basal synaptic transmission

Synthetic Aβ (500 nM), did not affect baseline excitatory synaptic transmission, EPSP's measuring 99±3% of control after 40 min perfusion ($p>0.05$, $n=10$) (Fig 3.2.). However a ten hours perfusion of Aβ at 2 μM at room temperature induced a small inhibition of baseline EPSP measuring 82±2% (Fig.3.3). Previous studies on the effect of Aβ on NMDAR is under debate. Wu et al (1995) reported that Aβ can up-regulate NMDAR function in the dentate gyrus, but in another work(Raymond et al., 2003), fEPSP of NMDA was inhibited by Aβ. To test whether the inhibition of LTP by Aβ is due to the block of NMDAR, we determined the effect of Aβ on NMDAR function by isolating NMDAR-mediated fEPSPs through perfusion by an ACSF without Mg^{2+} , plus 20 μM CNQX to block AMPA receptors. In control slices, NMDA-mediated fEPSPs could be recorded for 120 min, and application of 50 μM D-APV completely abolished the responses(data

not shown), confirming that they were mediated by NMDARs. Perfusion of Aβ resulted in a small but significant inhibition of NMDAR potential (Fig. 3.4, P<0.01). The NMDA field EPSP measured 79±5% of baseline at 90min after Aβ perfusion.

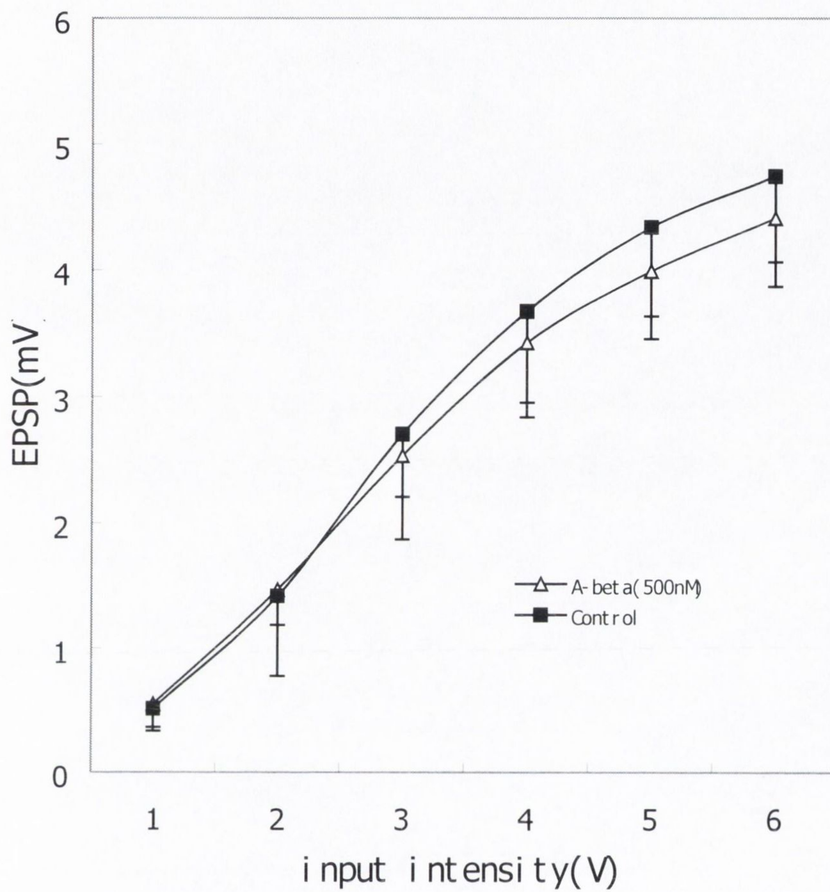

Fig 3.1 Effect of on input-output curve. Graphs show perfusion with synthetic (open triangles, n=8) has no effect on the baseline input-output curve(filled cycle, n=8).

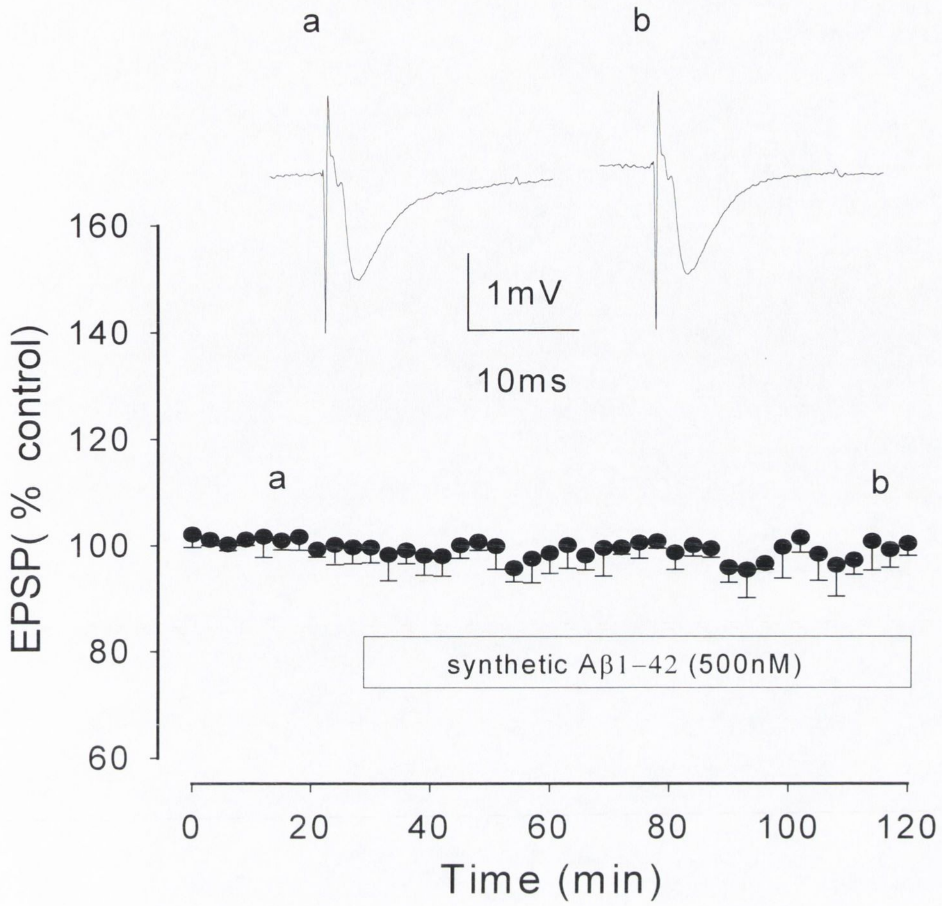

Fig 3.2 Administration of 500 nM synthetic Aβ has no effect on baseline EPSPs(n=5)

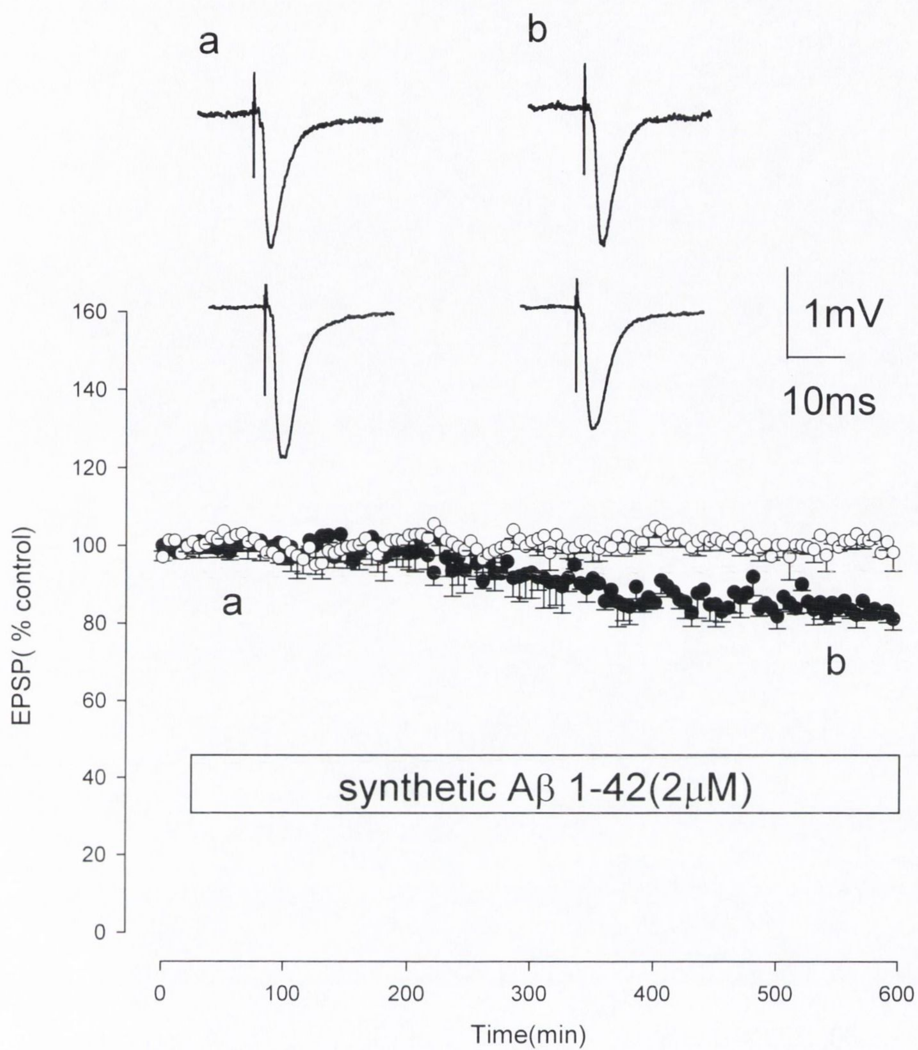

Fig 3.3 A longer time perfusion with higher concentration Aβ inhibited the baseline transmission by 20 %(closed circles, n=4), significantly reduced from control. (open circles, n=3).

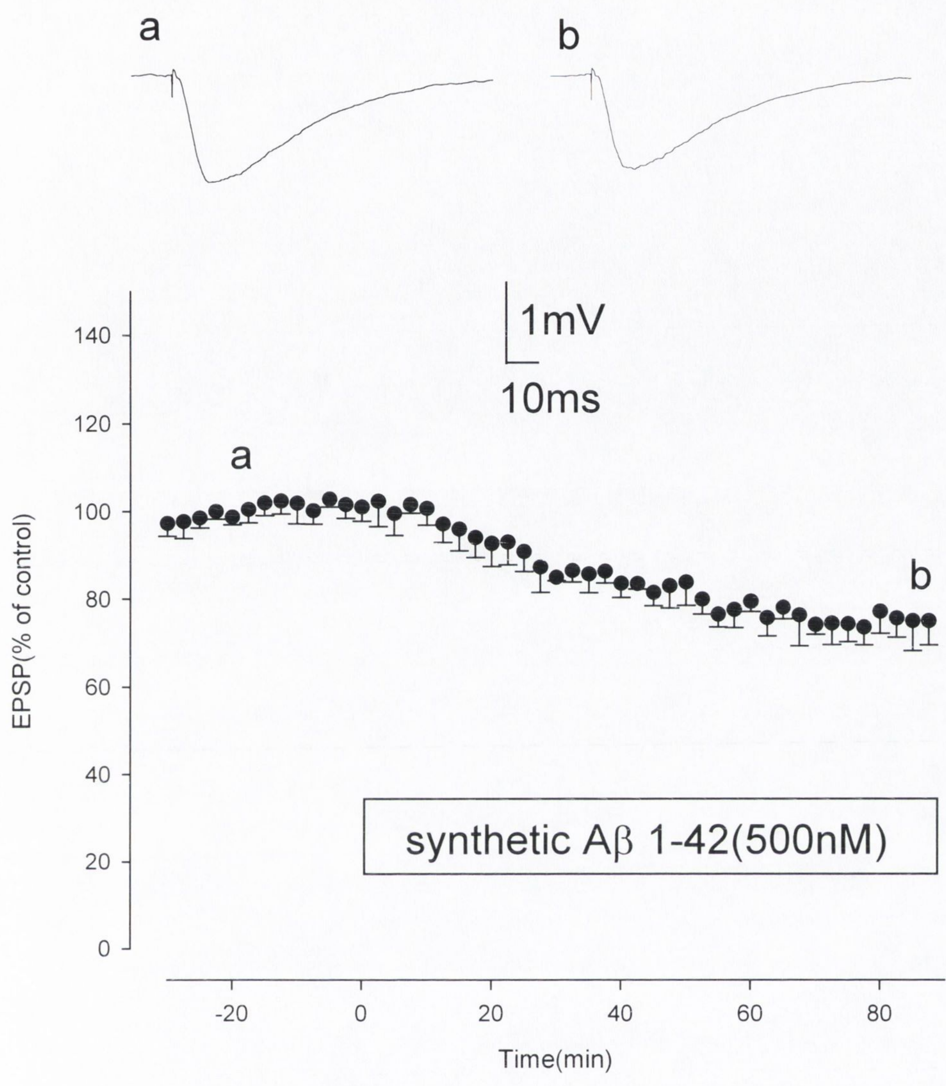

Fig.3.4 Aβ was applied for 90 min, causing a slight inhibition of NMDA potentials(n=5). Inset, representative NMDAR-mediated fEPSPs recorded prior to and 90 min after the administration of Aβ.

3.3 Effect of on Aβ synaptic plasticity

3.3.1 Synthetic Aβ inhibits induction of NMDAR-dependent LTP

In rat hippocampal slices, HFS induced NMDAR-dependent LTP under control conditions that reached a peak amplitude of ~100% above baseline immediately following HFS and then slowly declined over the next hour to 50-80% above baseline. The averaged LTP measured 201±15%, 166±12% and 157±5% at peak, 20 and 60 min post-HFS respectively ($p<0.005$, $n=55$, Fig 3.5).

Synthetic Aβ inhibited the induction of LTP, including the early and late phases of LTP. In the presence of synthetic $A\beta_{1-42}$ (500 nM), perfused for 60 min prior to HFS, LTP measured 151±13%, 110±8% and 104±7% at peak, 20 min and 60 min post-HFS respectively, all three values significantly less than control ($p<0.005$, $n=38$) (Fig 3.5). A two-way ANOVA comparing LTP in control and in Aβ also showed significant inhibition ($F= 48.2$, $p < 0.001$).

200 nM synthetic Aβ also inhibited LTP induction, which measured 176±8%, 131±6% and 115±7% at peak, 20 min and 60 min post-HFS respectively ($p<0.05$, $n=5$) (Fig3.6). However, 100 nM Aβ did not significantly inhibit LTP induction, LTP measuring 192±23, 155±18 and 146±10 at peak, 20 min and 60 min post-HFS respectively ($p>0.05$, $n=5$) (Fig3.6).

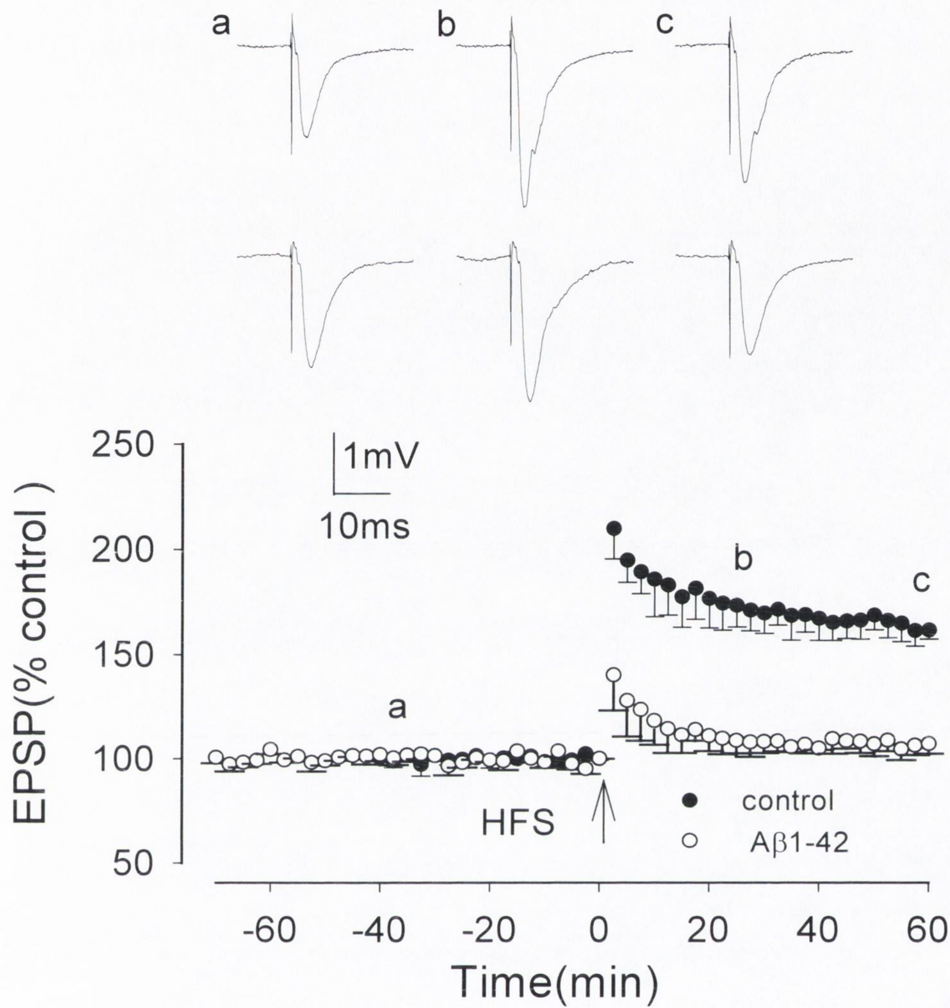

Fig.3.5 NMDAR-dependent LTP induced by a single brief HFS in the medial perforant path of the dentate gyrus in control (filled circles, n=10) and in the presence of synthetic Aβ (500 nM), applied 40 min prior to HFS (open circles, n=10), significantly reduced from control. The traces a, b and c illustrate EPSPs prior to, and 20 and 60 min after HFS respectively.

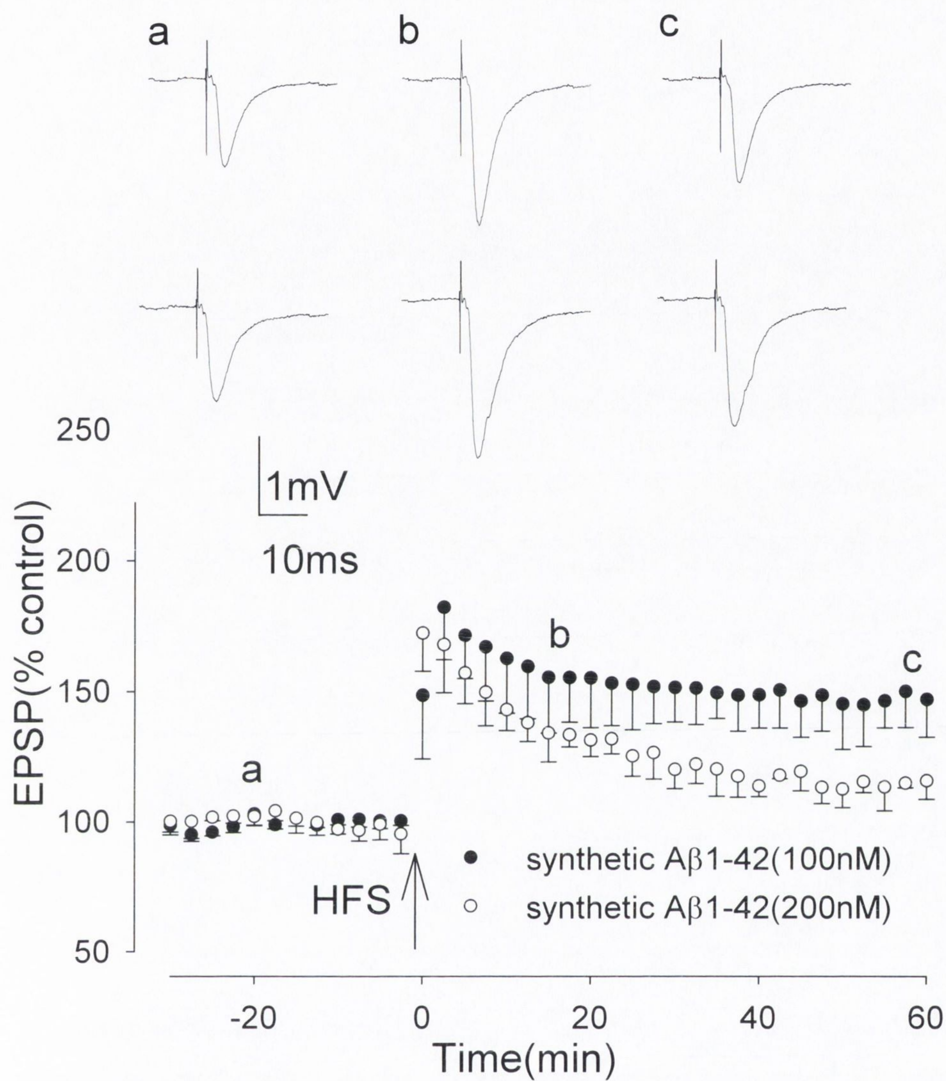

Fig 3.6 200nM(n=5) synthetic Aβ 1-42 but not 100 nM(n=5) inhibited the induction of LTP.

3.3.2 Aβ does not inhibit the expression of NMDAR-dependent LTP

In order to investigate the effect of Aβ on the expression of LTP, synthetic Aβ was perfused immediately following HFS. The expression of LTP following a single HFS was not inhibited by Aβ, LTP measuring 163±8% at 60 min post-HFS ($P>0.05$, $n=4$)(Fig 3.7).

3.3.3 Aβ does not inhibit induction of NMDAR-independent LTP or LTD

In order to determine whether the inhibitory action of Aβ was confined to NMDAR-dependent LTP or affected other types of plasticity, the effect of Aβ was investigated on NMDAR-independent LTP and LTD. Although NMDAR-independent LTP can be induced by strong stimulation in the dentate gyrus (Wu et al, 2004), such stimulation commonly results in induction of a group I mGluR-dependent LTD (Wu et al, 2004). In the present study, we therefore choose to study NMDAR-independent LTP in CA1, a region in which NMDAR-independent LTP has been intensively studied and found to be dependent on activation of mGluR and voltage-gated Ca channels (Grover and Teyler, 1990; Cavus and Teyler, 1996; Grover, 1998; Wu et al, 2004). Aβ has been previously shown to block induction of NMDAR-dependent LTP in CA1 in a manner identical to that in the dentate gyrus (Cullen et al, 1997; Walsh et al, 2002, Itoh et al, 1999; Chen et al, 2000; Vitolo et al, 2002).

Aβ (1 μM) did not inhibit NMDAR-independent LTP, recorded in the presence of 100 μM D-AP5 to prevent NMDAR activation. In control, HFS induced NMDAR-independent LTP measuring 158±16% and 136±7% at peak and at 60 min post-HFS respectively, significant LTP ($p<0.005$, $n=15$, Fig 3.8). In the presence of Aβ, perfused for 40 min prior to HFS, LTP measured 144±15% and 129±3% at peak and 60 min post-HFS respectively, values not significantly reduced from control ($p>0.05$, $n=5$). A two-way ANOVA also showed no significant inhibition ($F=4.6$, $p=0.076$).

We also investigated the effect of Aβ on a further form of plasticity, that of LTD. Induction of LTD by LFS (900 stimuli at 1Hz) was not affected by Aβ, with LTD at 60 min post-LFS measuring 23±3% in control and 21±2% after Aβ perfusion for 40 min ($p>0.05$, $n=5$, Fig 3.9). A two-way ANOVA also showed no significant inhibition ($F=4.3$, $p=0.071$).

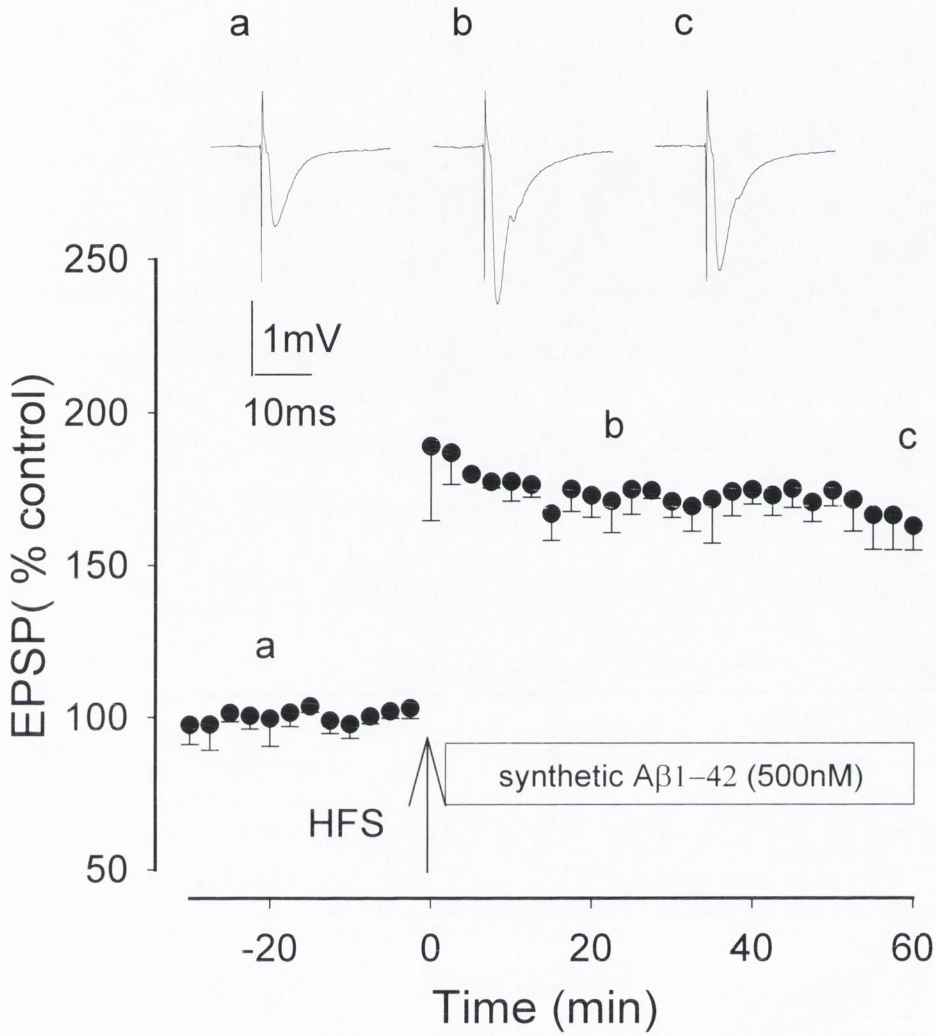

Fig 3.7 LTP in experiments in which synthetic Aβ was applied immediately following HFS, LTP expression not being significantly reduced from control(n=5).

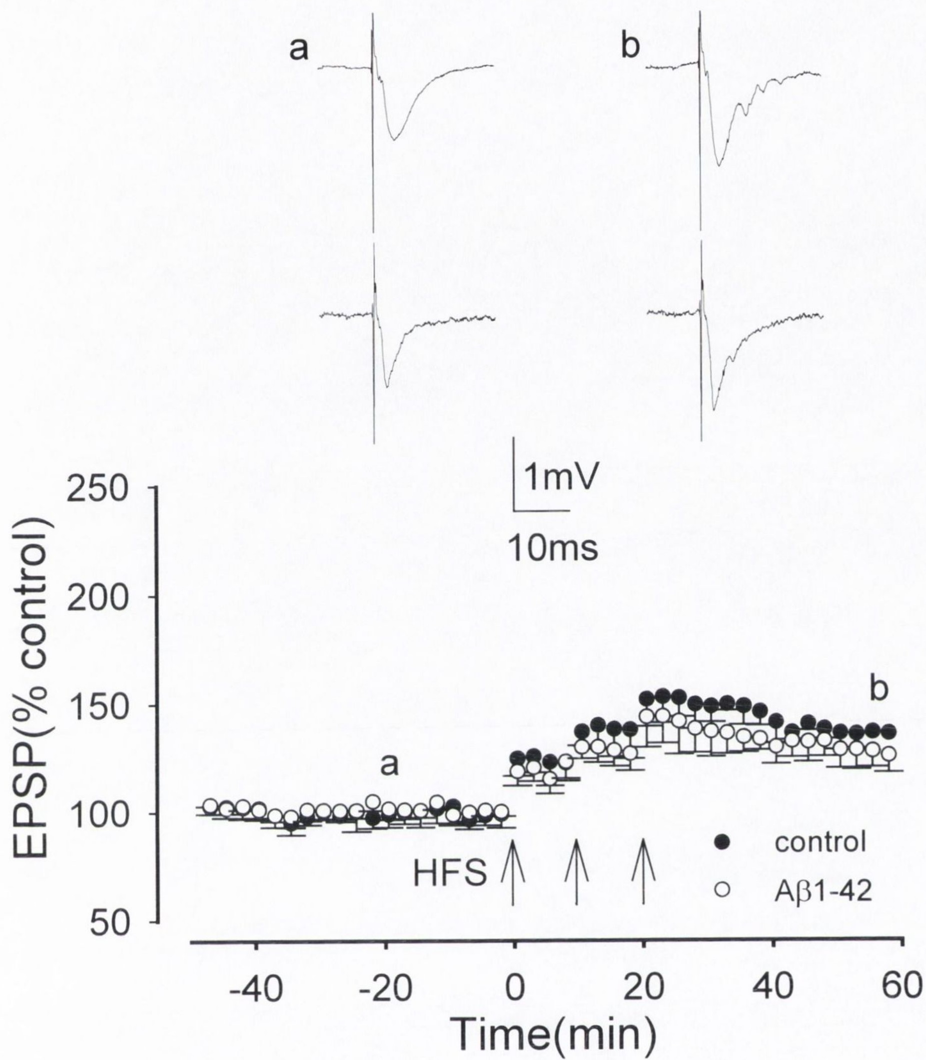

Fig.3.8 NMDAR-independent LTP induced by a single brief HFS in CA1 in control (filled circles, (n=5)) and in the presence of synthetic Aβ (1μM), applied 40 min prior to HFS (open circles, (n=5)), not significantly reduced from control.

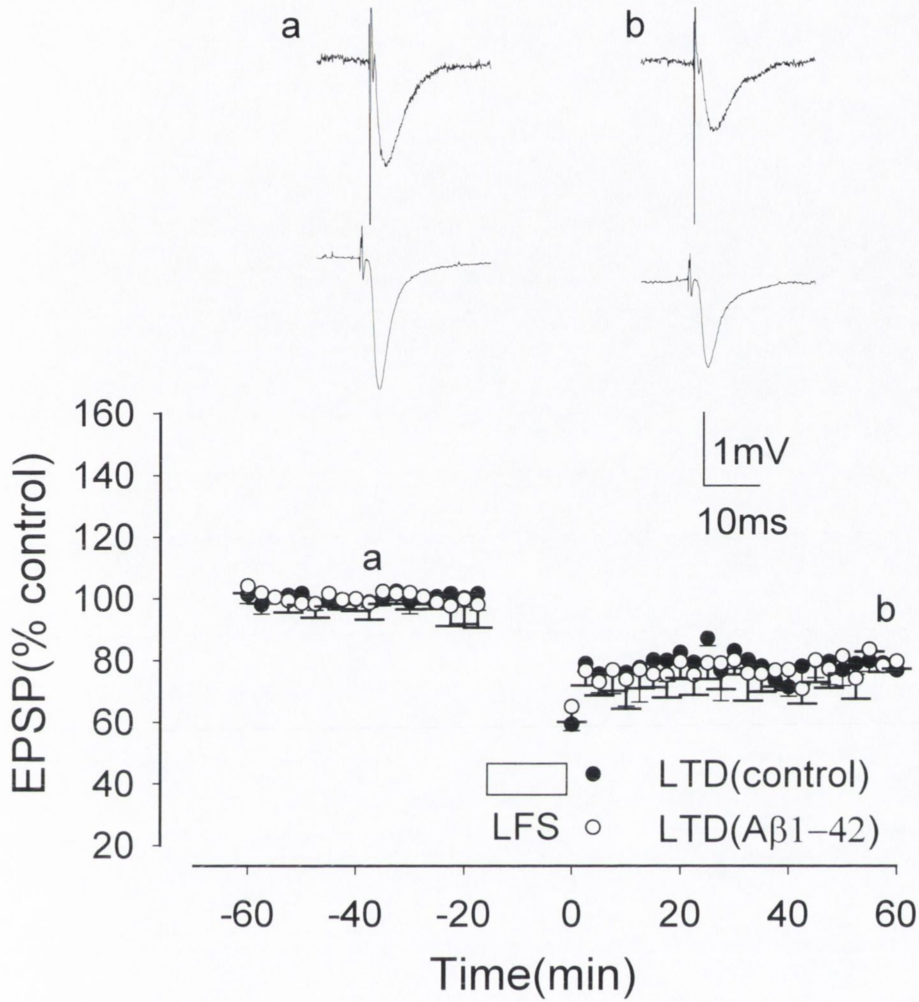

Fig.3.9 LTD induced by LFS (1Hz, 15 min) in the dentate gyrus in control (filled circles, n=5), and in the presence of Aβ (500 nM, n=5), not significantly reduced from control.

3.4 Effect of human cell-derived Aβ on NMDAR-independent LTP

Synthetic Aβ 1-42 assembles into a variety of structures in aqueous buffers, including low n-oligomers, ADDLs, protofibrils and fibrils (Walsh et al, 1997, Lambert et al, 1998; Bitan et al, 2002). The solutions of synthetic Aβ used in this study contained a mixture of these assemblies. To determine if soluble forms of Aβ contributed to the inhibition of LTP in vitro, we tested the effect of 7PA2 conditioned medium (CM) which contains only Aβ monomer and low n-oligomers, and is free of fibrils and protofibrils (Walsh et al. 2002). Conditioned medium containing cell-derived Aβ was collected from 7PA2 cells. CHO-CM, which does not contain human Aβ, was used as a negative control (see Methods). The medium from the cell cultures was diluted with DMEM prior to perfusion onto the slices.

Control LTP in CHO-CM devoid of cell derived Aβ was not significantly different from LTP in physiological medium, measuring 223±18%, 192±13% and 188±10% at peak, 20 min and 60 min post-HFS respectively (Fig 3.10, n=5). However, in the presence of cell-derived naturally secreted Aβ, diluted three-fold in DMEM (measured Aβ concentration was 1.1 nM) and perfused for 60 min prior to HFS, LTP induction was inhibited, measuring 170±10%, 127±6% and 110±7% at peak, 20 min and 60 min post-HFS respectively. All three values were significantly less than control LTP in normal bath media or CHO-CM ($p<0.005$) (Fig 3.10, n=5). Cell-derived Aβ diluted five-fold also inhibited LTP induction, although to a lesser extent than the 3-fold

dilution, measuring 192±11%, 153±5% and 135±7% at peak, 20 min and 60 min post-HFS respectively, ($n = 5$, $p<0.01$) (Fig3.11).

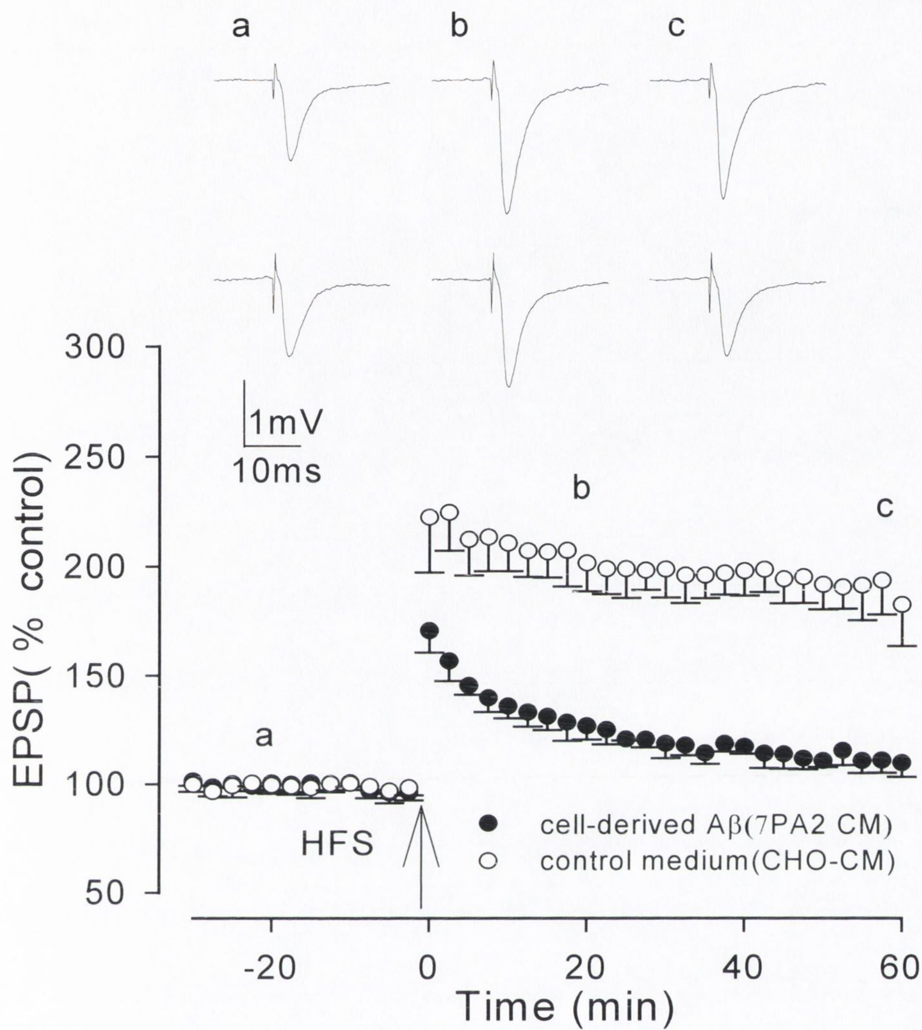

Fig 3.10 Control LTP induction in CHO-CM (open circles, n=5) and LTP induction in the presence of 7PA2-CM containing cell-derived Aβ (filled circles, n=5), applied 60 min prior to HFS, significantly reduced from control.

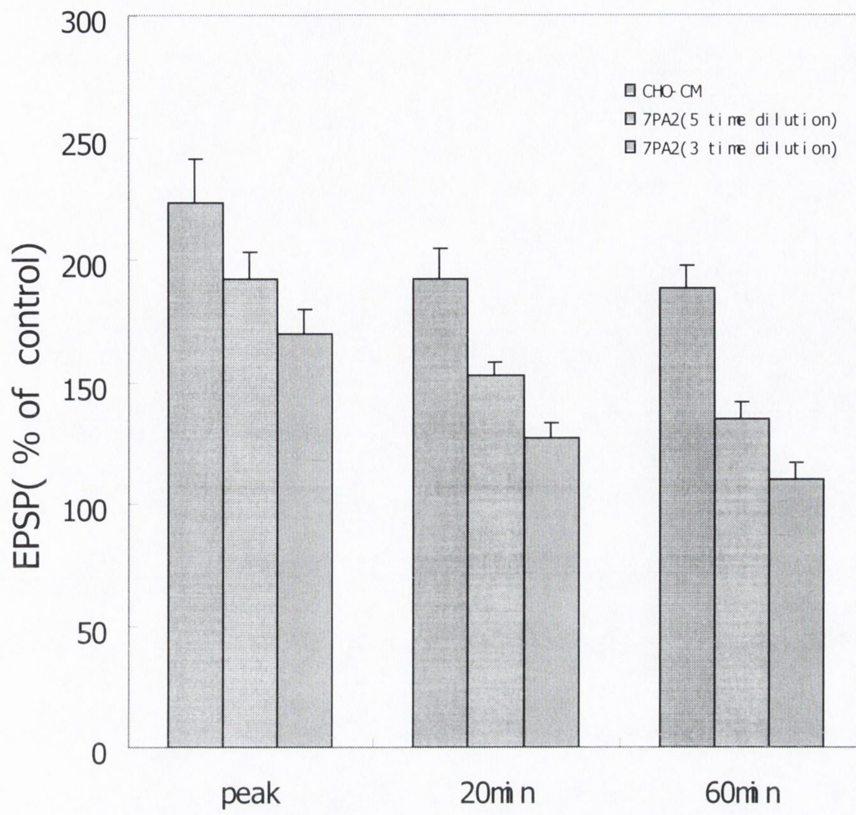

Fig 3.11 The inhibitory effect of different concentrations of cell-derived Aβ on the induction of LTP.

3.5 Effect of kinases inhibitors on Aβ – induced impairment LTP

3.5.1 The inhibition of LTP by synthetic and cell-derived Aβ is prevented by inhibitors of JNK

The JNK group of protein kinases are a subgroup of the MAP kinase family which are known to be activated by cellular stresses, including synthetic Aβ (Bozyzcko-Coyne et al, 2001; Morishima et al, 2001; Troy et al, 2001). Activation of JNK occurs in the AD brain (Shoji et al, 2000; Zhu et al, 2001a). In order to assess the involvement of JNK in the inhibitory effects of Aβ on LTP induction, two JNK inhibitors, JNKI and SP600125, were studied. The inhibitor JNKI, based on amino acids 143-163 of the JNK-binding domain of the JNK scaffolding protein, JNK-interacting protein-1, has been shown to interact directly and inhibit JNK (IC_{50} = ~0.5 μM), but does not inhibit the activities of the related ERK and p38 MAPKs (Bonny et al, 2001; Barr et al, 2002).

LTP induction was not altered by JNKI (2 μM) perfused alone, measuring 198±11%, 150±4% and 138±9%, at peak, 20 min and 60 min post-HFS respectively (n=5, $p>0.05$) (Fig 3.12). These values of LTP in JNKI are not significantly different from those of control LTP in the normal physiological medium. However, JNKI prevented the inhibition of LTP by synthetic Aβ, LTP measuring 178±12%, 147±7% and 136±5%, n=6, at peak, 20 min and 60 min post-HFS respectively, values significantly greater than the values of LTP induction in the presence of synthetic Aβ alone ($p<0.01$) and not significantly different from control

LTP ($p>0.05$) (Fig 3.12). JNKI also prevented the inhibition of LTP induction by cell-derived Aβ, LTP measuring 200±14%, 154±7% and 140±7%, n=5, at peak, 20 min and 60 min post-HFS respectively in the presence of JNKI, values significantly increased from those in the presence of cell-derived Aβ alone ($p<0.01$), and not significantly different from control LTP in CHO-CM ($p>0.05$) (Fig 3.13 , n=5).

To confirm these results, a second JNK inhibitor was examined. SP600125 is a potent (Ki = 0.19 μM) ATP-competitive JNK inhibitor based on an anthrapyrazalone series with a >20 fold selectivity over a range of other kinases, including ERK, p38, PKA and PKC (Bennett et al, 2001). SP600125 (20μM), perfused alone, did not significantly alter LTP induction, which measured 215±17%, 176±10% and 161±5%, at peak, 20 min and 60 min post-HFS respectively (n=5, $p>0.05$) (Fig 3.14). LTP induction was also not altered by 0.1% DMSO alone, the vehicle used for SP600125 and certain other compounds (see Methods), LTP measuring 203±11%, 164±17% and 152±13% (n=5, $p>0.05$) at peak, 20 min and 60 min post-HFS respectively (data not shown). Similar to JNKI, SP600125 prevented the inhibition of LTP by synthetic Aβ, LTP measuring 180±6%, 137±2% and 133±2%, at peak, 20 min and 60 min post-HFS respectively, values significantly increased from the values of LTP induction in the presence of synthetic Aβ alone (n=5, $p<0.05$) (Fig 3.14). However, at the concentration used (20 μM), SP600125 did not completely reverse the inhibition of LTP by Aβ, the values of LTP induction in SP600125 and Aβ being significantly lower than in SP600125 alone ($p<0.05$).

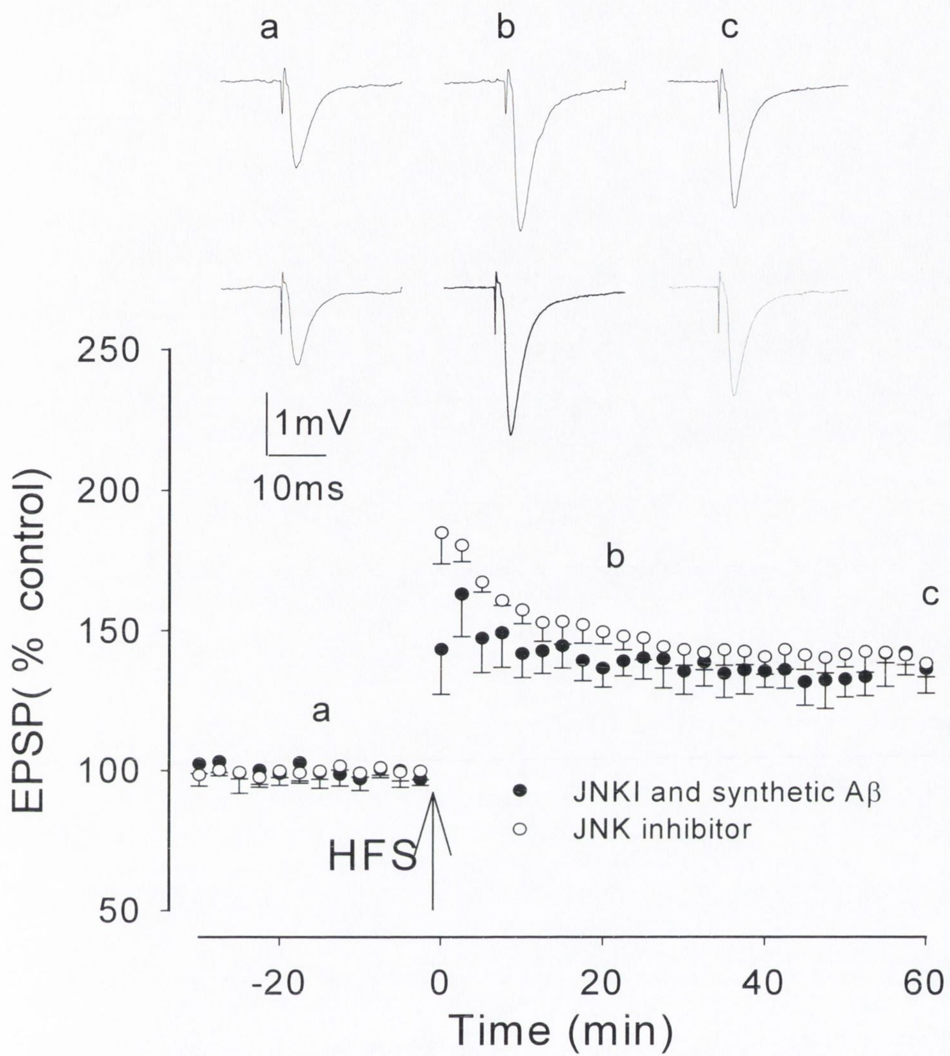

Fig 3.12 LTP induction in JNKI (open circles, n=5) and in 500 nM synthetic Aβ plus JNKI (filled circles, n=5), not significantly inhibited.

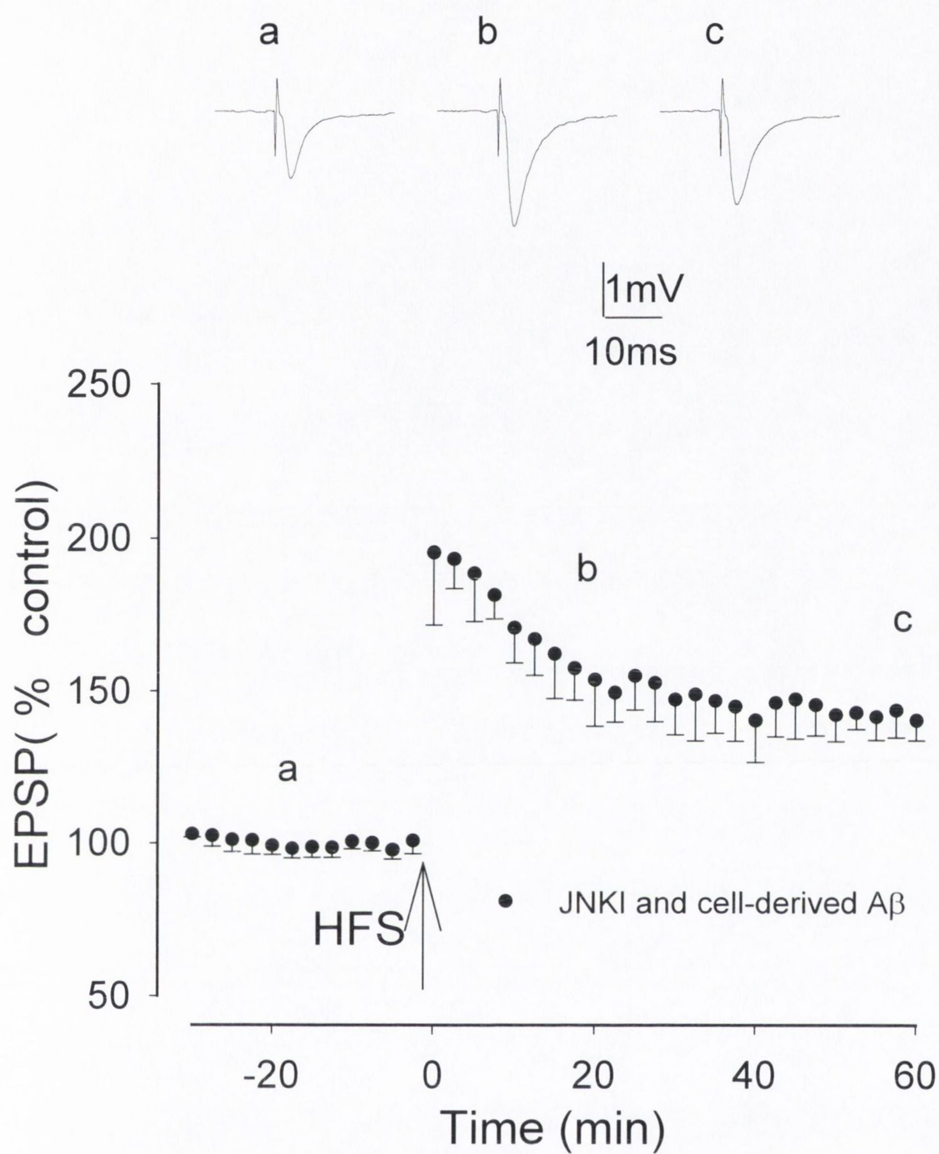

Fig 3.13 LTP induction in naturally secreted cell-derived human Aβ plus JNKI, not significantly inhibited (n=5).

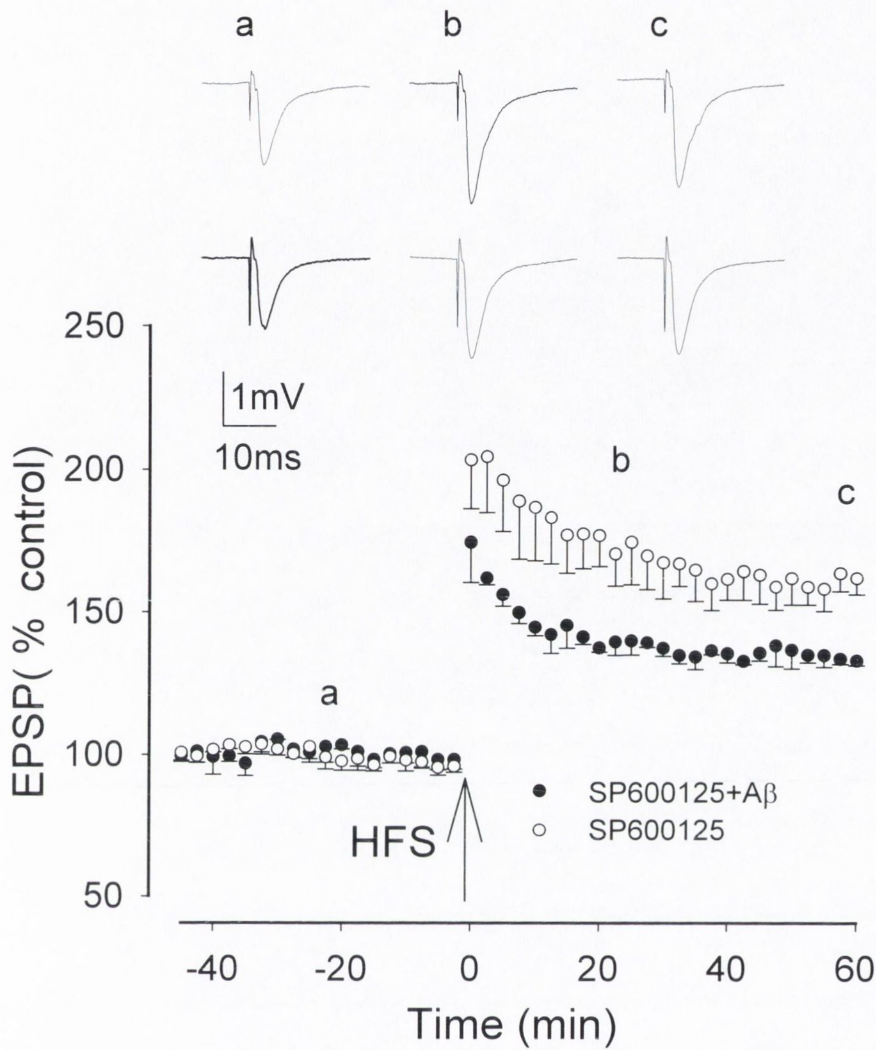

Fig 3.14 LTP induction in the JNK inhibitor SP600125 (open circles, n=5) and in synthetic Aβ plus SP600125 (filled circles, n=5), significantly increased from the inhibited LTP induction in Aβ.

3.5.2 The inhibition of LTP by synthetic Aβ is prevented by inhibitors of Cdk5

Cdk5 is a Ser-Thr kinase which has important general roles in phosphorylating cell cycle and cytoskeletal proteins. Activation of Cdk5 by binding to its regulatory subunit p35 to form the p35/Cdk5 complex is essential for neuronal development and plasticity (Dhavan and Tsai, 2001). There is compelling evidence that inhibition of the activity of Cdk is detrimental to survival of neurons (Nygen et al, 2002), and Cdk5 has been implicated in Alzheimer's pathology (Ahlijanian et al, 2000; Patrick et al, 1999). In order to determine the involvement of Cdk5 in the inhibitory effects of Aβ on LTP induction, two inhibitors of Cdk5, butyrolactone and roscovitine, were studied. Butyrolactone and roscovitine are potent inhibitors of Cdk5, with an IC_{50} of 0.49 μM (Liu et al, 2001) and 0.16 μM (Knockaert et al, 2002) respectively.

Both butyrolactone and roscovitine reversed the inhibitory effect of synthetic Aβ on LTP induction. Butyrolactone alone, perfused 60 min prior to HFS, did not alter LTP induction, which measured 192±9%, 160±7% and 147±9% at peak, 20 min and 60 min post-HFS respectively, values not significantly different from control LTP (n=6, $p>0.01$) (Fig3.15). However, butyrolactone prevented the inhibition of LTP induction by synthetic Aβ. Thus LTP induction measured 184±10%, 145±6% and 136±6% at peak, 20 min and 60 min post-HFS respectively in the presence of butyrolactone, values significantly increased from those in the presence of synthetic Aβ alone ($P<0.05$), and not significantly different from control LTP (n=6, $p>0.05$)(Fig 3.15). Roscovitine alone, perfused from 60 min prior to HFS, did not alter LTP

induction, which measured 194±20%, 159±10% and 150±5% at peak, 20 min and 60 min post-HFS respectively, values not significantly different from control LTP ($P>0.01$, n=6) (Fig 3.16). However, roscovitine prevented the inhibition of LTP induction by synthetic Aβ, LTP induction measuring 176±16%, 142±7% and 142±4%, n=6 at peak, 20 min and 60 min post-HFS respectively, values significantly increased from the values of LTP induction in the presence of synthetic Aβ alone ($P<0.05$), and not significantly different from LTP in roscovitine alone ($P>0.05$)(Fig 3.16).

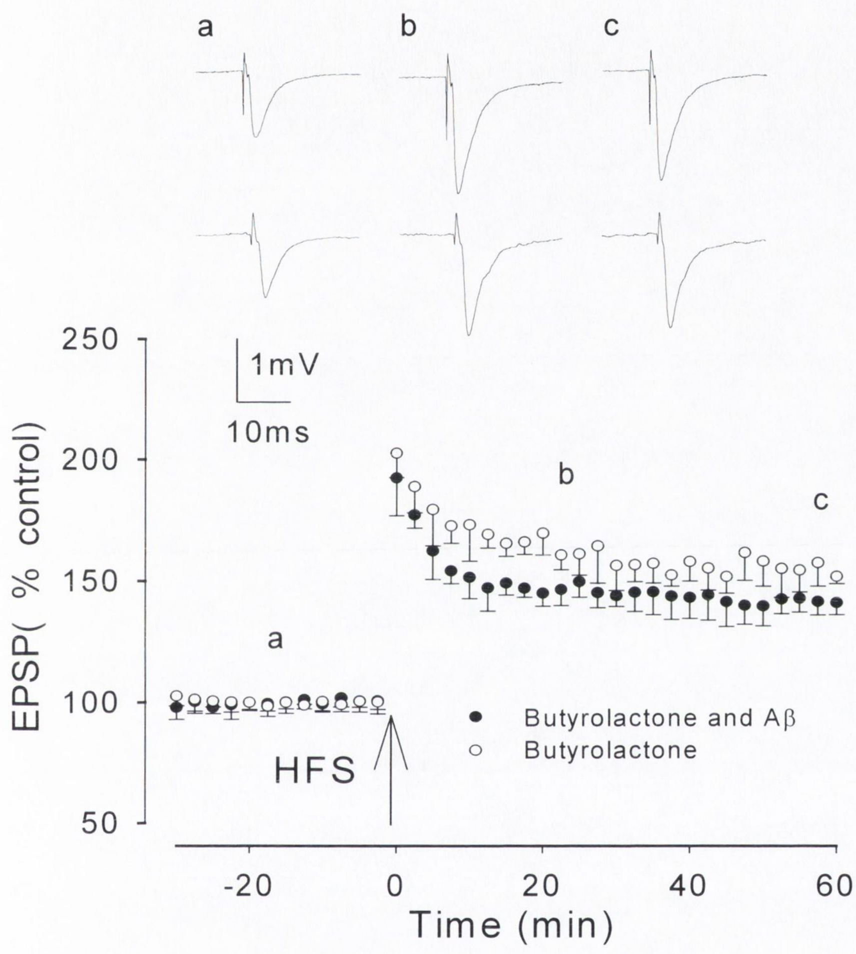

Fig 3.15 LTP induction in butyrolactone (open circles, n=5) and in the presence of butyrolactone plus 500 nM synthetic Aβ (filled circles, n=5), not significantly inhibited.

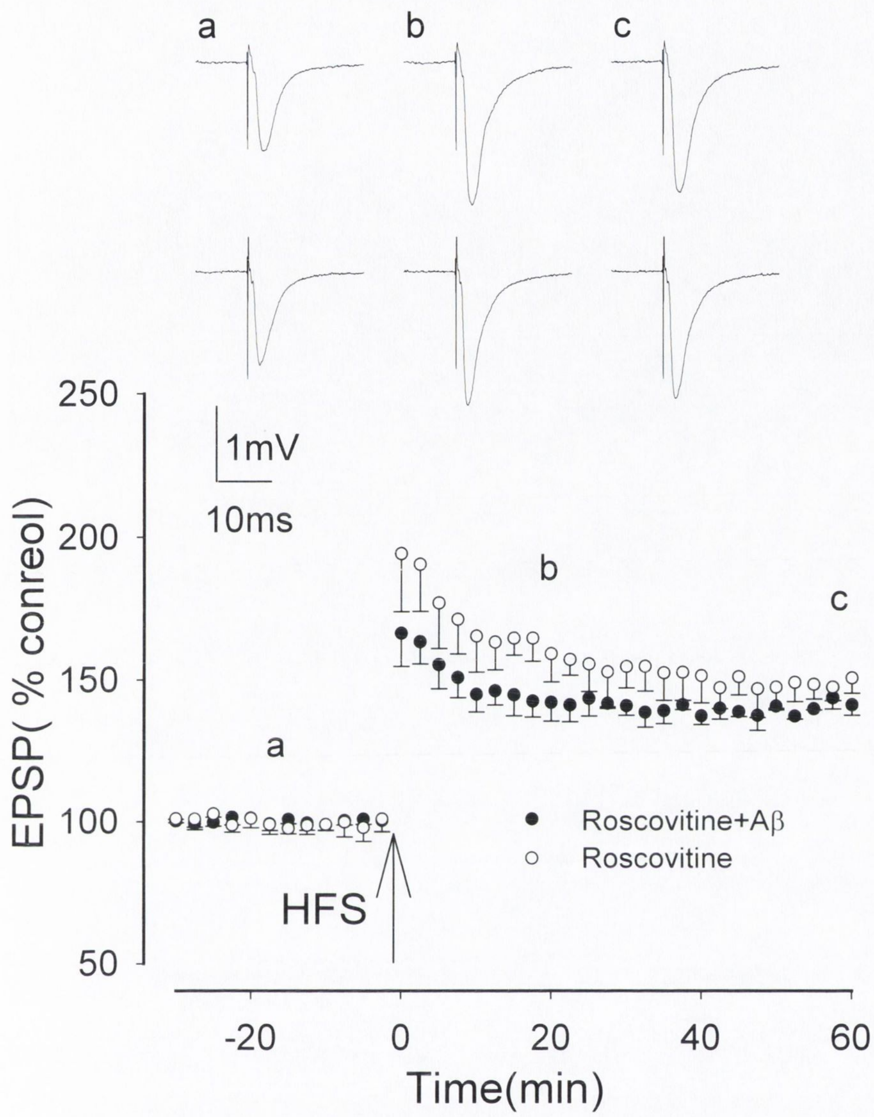

Fig 3.16 LTP induction in roscovitine (open circles, n=6) and in the presence of roscovitine plus 500 nM synthetic Aβ (filled circles, n=5), not significantly reduced from control.

3.5.3 The inhibition of LTP by synthetic Aβ is prevented by inhibition of p38 MAP kinase but not p42/44 MAP kinase

P38 MAPK and p42/44 MAPK are distinct subgroups of the MAP kinase family. The p38 MAPK subgroup is well known to be involved in inflammation and cell death (Ono and Han, 2000), while p42/44 MAPKs have been extensively characterised as a central component of signal transduction pathways stimulated by growth-related stimuli. In order to determine if the inhibitory effects of Aβ on LTP induction are mediated via activation of p38 and/or p42/44 MAPKs, the effect of applying synthetic Aβ in the presence of inhibitors of these kinases was determined.

SB 203580 is a highly selective p38 MAP kinase inhibitor with an IC_{50} of 34 nM (Lee et al, 1994). SB203580 (1 μM) applied alone did not alter LTP induction, which measured 192±9%, 160±7% and 147±9% at peak, 20 min and 60 min post-HFS respectively, values not significantly different from control LTP ($n=6$, $p>0.005$) (Fig 3.17). However, SB203580 prevented the inhibition of LTP induction by synthetic Aβ. Thus LTP induction measured 184±10%, 145±6% and 136±6%, at peak, 20 min and 60 min post-HFS respectively in the presence of SB203580, values significantly increased from the values of LTP induction in the presence of synthetic Aβ alone ($n=6$, $p<0.005$), and not significantly different from control LTP ($p>0.05$)(Fig 3.17).

The effect of inhibition of p42/44 MAP kinase was investigated using the MAP kinase kinase (MEK) inhibitor UO126 (5 μM). This concentration of U0126 is known to completely block both basal and stimulus-induced activation of p42/44 MAP kinase in hippocampal slices (Robertson et al, 1999). UO126 applied alone did not alter LTP induction,

which measured 207±12%, 172±13% and 146±7% at peak, 20 min and 60 min post-HFS, values not significantly different from those of control LTP induction (n=5, p>0.05) (Fig 3.18). UO126 did not prevent the inhibition of LTP by Aβ, LTP measuring 152±12%, 106±6% and 97±3% at peak, 20 min and 60 min post-HFS, values not significantly different from those in Aβ alone (p>0.05) (Fig 3.18).

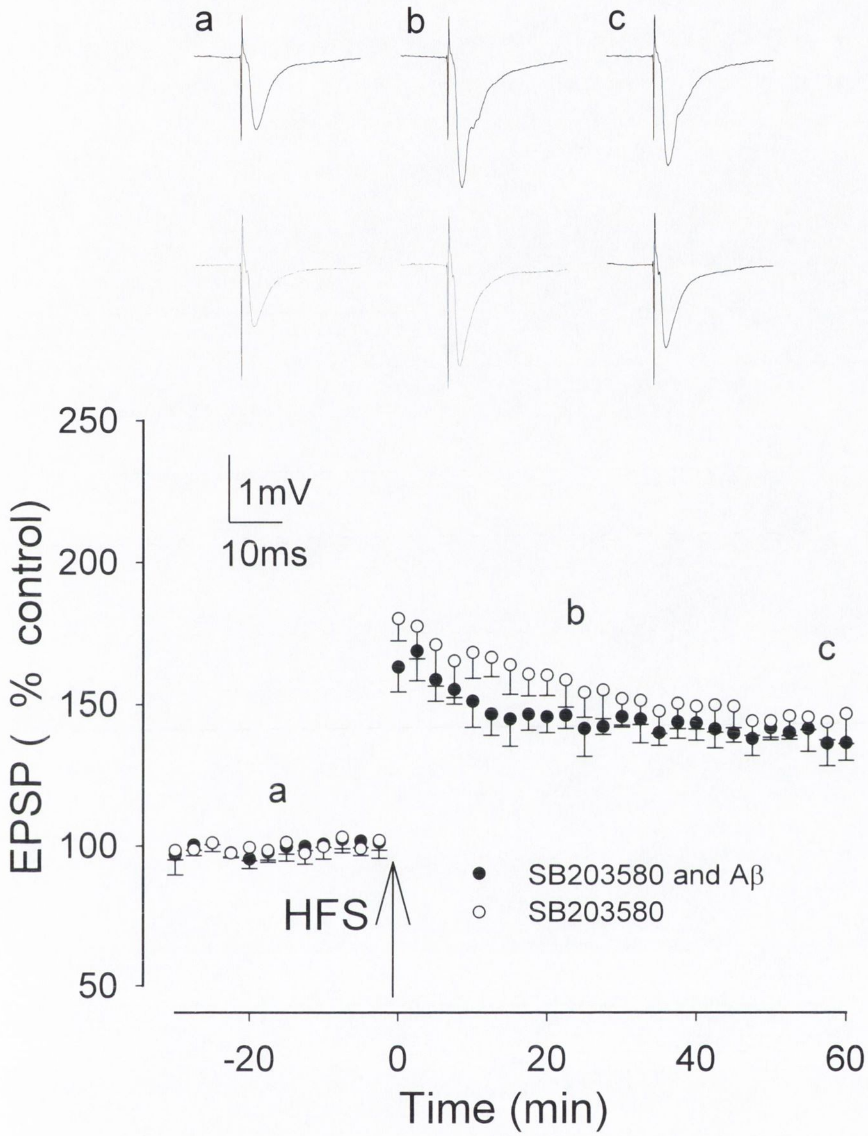

Fig 3.17 LTP induction in SB203580 (open circles, n=5), and in 500 nM synthetic Aβ plus SB203580 (filled circles, n=5), not significantly reduced from control.

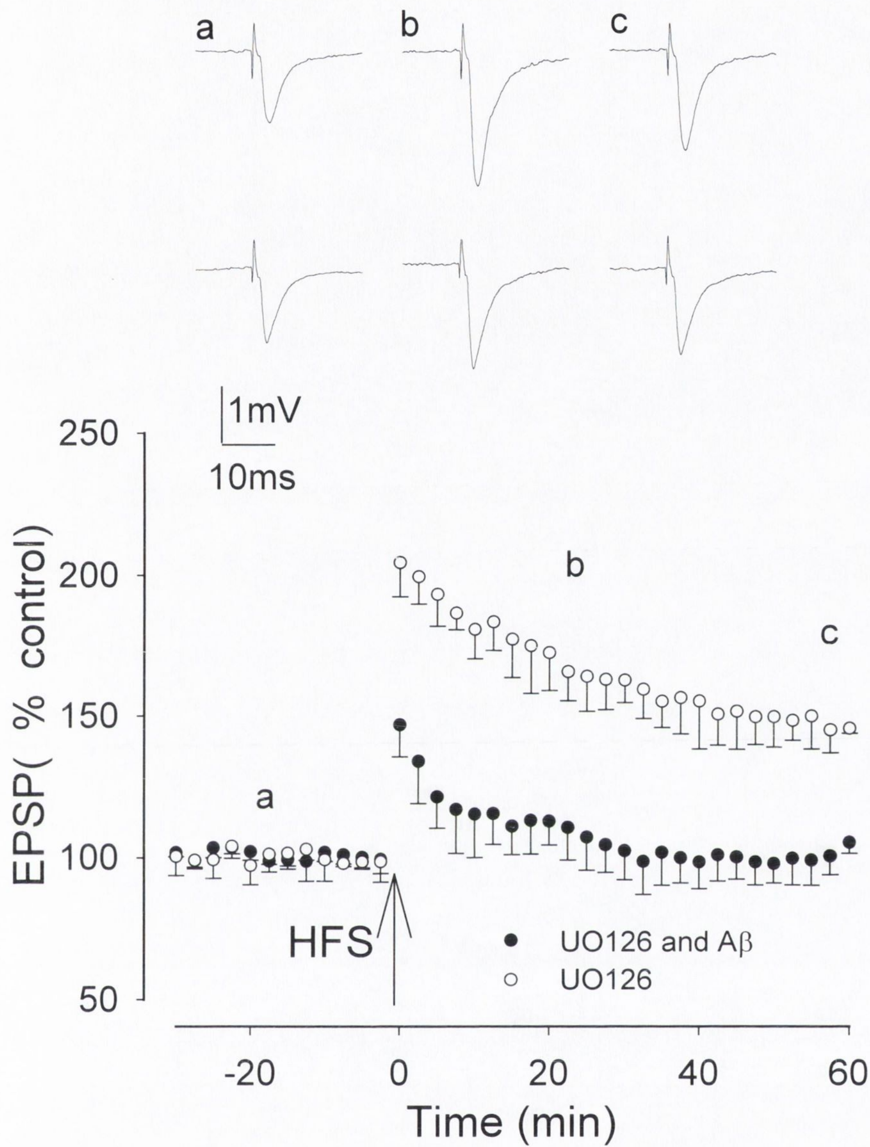

Fig 3.18 LTP induction in UO126 (open circles, n=5), and in 500 nM synthetic Aβ plus UO126 (filled circles, n=5), significantly reduced from control.

3.6 Inhibition of microglia activation prevents Aβ inhibition of LTP

Several neurochemical studies have reported that the neurotoxic effects of Aβ are mediated through activation of microglia (McDonald et al, 1997; Minghetti and Levi, 1998; Tan et al, 1999). Minocycline is a tetracycline derivative that has powerful anti-inflammatory effects that are not due to its antimicrobial action but rather due to inhibition of microglial activation (Yrjanheikki et al, 1998, Tikka et al, 2001; Zhu et al, 2002). In order to determine if the Aβ-mediated inhibition of LTP induction involves activation of microglia, we have determined if minocycline prevents such Aβ-mediated LTP inhibition.

Minocycline was perfused for 60 min prior to HFS. Minocycline (20 μM) perfused alone, did not alter LTP induction, which measured 204±19 and 149±11% at peak and 60 min post-HFS respectively ($p>0.05$, $n=5$, Fig 3.19). A two-way ANOVA showed no significant difference between LTP in control and in minocycline ($F=0.9$, $p=0.37$). However, minocycline reversed the Aβ inhibition of LTP induction, LTP measuring 186±23% and 145±1% at peak and 60 min post-HFS respectively in minocycline plus Aβ. These values were not significantly different from control values ($p>0.05$, $n=5$, Fig 3.19). A two-way ANOVA comparing LTP in control and in Aβ also showed no significant difference between LTP in minocycline and in minocycline plus Aβ ($F= 13.6$, $p>0.05$). A significant reversal of Aβ-mediated block of LTP, was also obtained using a lower concentration of minocycline (10 μM), LTP measuring 177±5, and 126±5% at peak and 60 min post-HFS respectively in

minocycline plus Aβ, values significantly increased from those in Aβ alone ($p<0.005$, $n=5$) (data not shown).

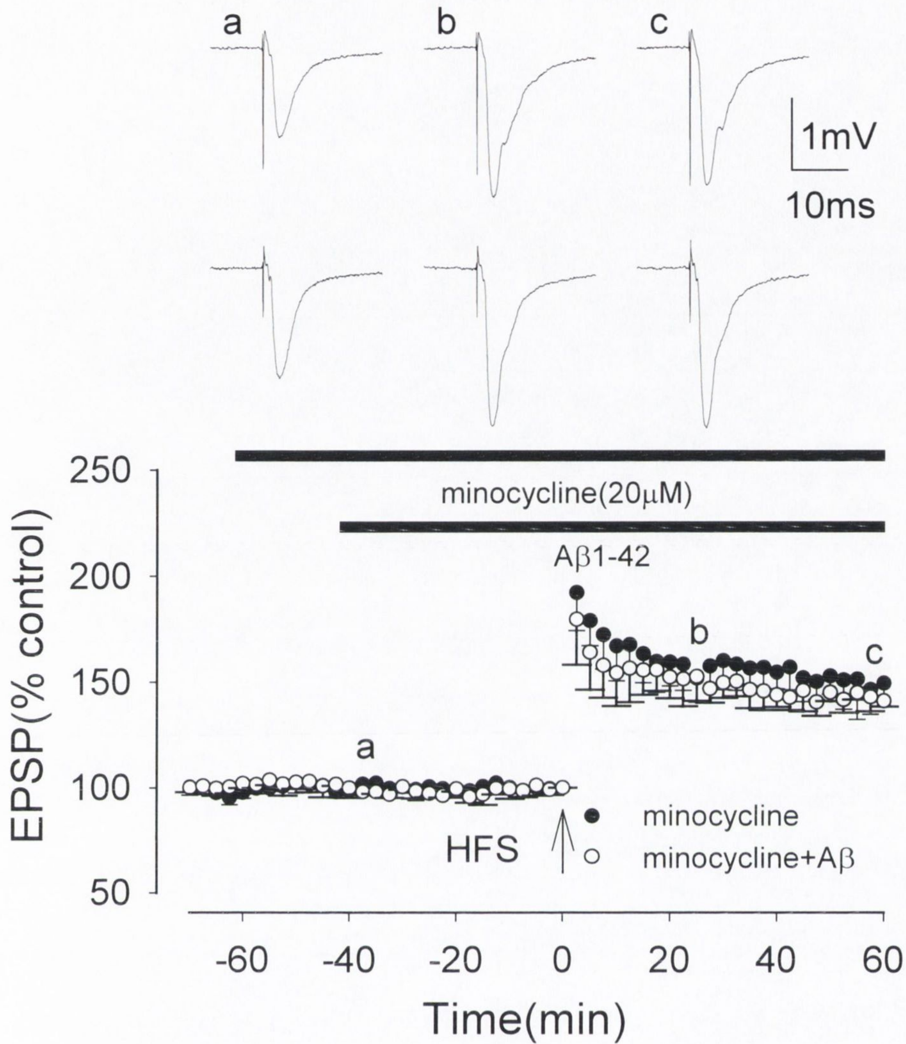

Fig.3.19 The graph shows the induction of LTP in the presence of minocycline (filled circles, n=5), not significantly different from control LTP, and in the presence of minocycline plus Aβ (open circles, n=5). Minocycline prevented the Aβ-mediated inhibition of LTP induction.

3.7 Effect of iNOS on Aβ – induced LTP impairment

3.7.1 Aβ does not inhibit LTP induction in iNOS knockout mice

Microglia are known to produce NO during inflammation by activation of the enzyme inducible nitric oxide synthase (iNOS) (Akama and Van Eldick, 2000), and increased levels of iNOS occur in cells surrounding amyloid plaques in the AD brain (Smith et al, 1997). Moreover, Aβ causes iNOS production in glia cells (Akama and Van Eldick, 2000). In the present study, we have investigated the role of iNOS in the Aβ-mediated inhibition of LTP induction by examining the effects of Aβ on LTP induction in iNOS mutant mice.

The effects of Aβ on LTP induction was first investigated in wild type mice. Control LTP induction in wild type mice was similar to that in juvenile rats, measuring 216±21% and 165±13% at peak and 60 min post-HFS respectively (significant LTP, $p<0.005$, n=15, Fig 3.20). Moreover, Aβ, perfused 45 min prior to HFS, inhibited LTP induction in a similar way to that observed in rats, with LTP measuring 163±7% and 110±16% at peak and 60 min post-HFS respectively ($p<0.005$, n=15, Fig 3.20). A two-way ANOVA comparing LTP in control and in Aβ also showed significant inhibition of LTP by Aβ ($F= 43.1$, $p < 0.001$). Control LTP in iNOS mutant mice was not significantly different from that in wild type mice, measuring 222±13% and 171±9% at peak and 60 min post-HFS respectively ($p>0.05$, n=5, Fig 3.21, F value = 1.5, P=0.26). However, in contrast to wild type mice, LTP induction was not inhibited by Aβ (500 nM), LTP measuring 216±18% and 152±11% at peak and 60 min post-

HFS respectively ($p>0.05$, $n=5$, Fig 3.21). A two-way ANOVA comparing LTP in the presence of Aβ in wild-type mice and iNOS mutant mice showed significant difference ($F= 40.7$, $p < 0.001$).

These experiments demonstrate a role for iNOS in the Aβ-mediated inhibition of LTP induction.

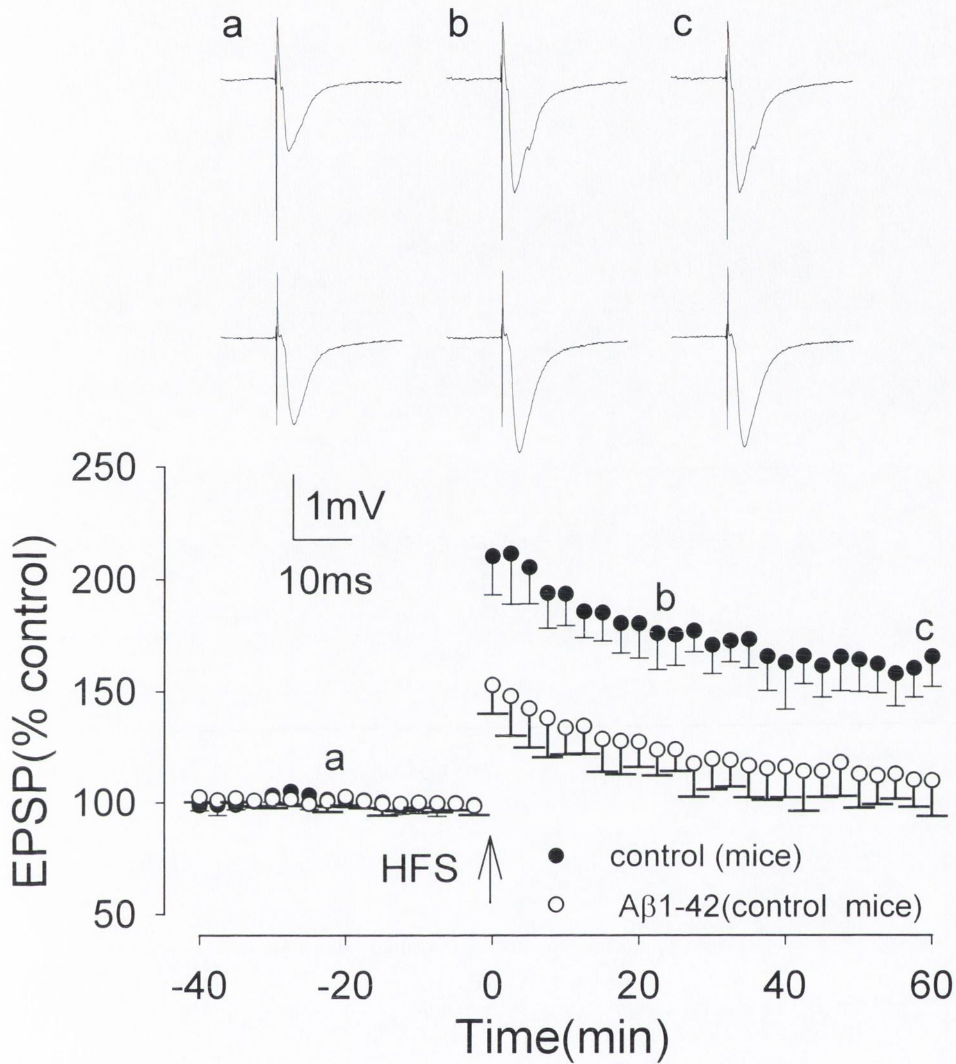

Fig.3.20 Induction of LTP in the medial perforant path of the dentate gyrus of wild type mice (filled circles, n=6) and in wild type mice plus Aβ (open circles, n=5). Aβ inhibited LTP induction in the mouse dentate gyrus to a similar extent to that in the rat dentate gyrus.

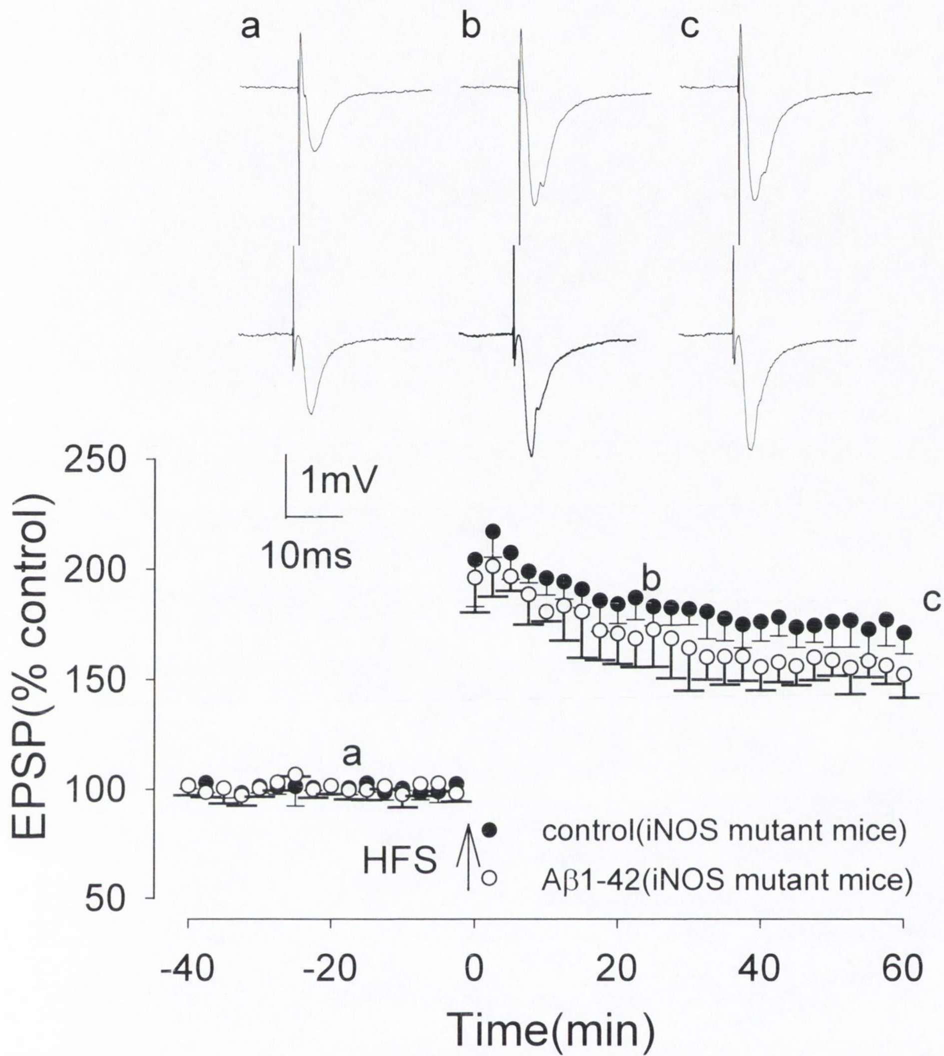

Fig.3.21 Induction of LTP in iNOS knockout mice (filled circles, n=5), not significantly different from wild type mice, and induction of LTP in the presence of Aβ in iNOS knockout mice (open circles, n=5), not significantly different to control.

3.7.2 Aβ-mediated inhibition of LTP induction is prevented by iNOS inhibitors

In order to further investigate the role of iNOS in the Aβ-mediated inhibition of LTP induction, the effect of NOS inhibitors were investigated on the Aβ-mediated inhibition of LTP in rat dentate gyrus. We first investigated the effects of aminoguanidine, which is known to inhibit both constitutive and inducible nitric oxide synthase isoforms (Lazlo et al, 1995). In control slices, aminoguanidine (100 μM) did not alter control LTP induction, which measured 221±14% and 170±3% at peak and 60 min post-HFS respectively ($p<0.05$, n=5, Fig 3.22, F value = 0.1, $p=0.76$). However, the Aβ-mediated inhibition of LTP induction was prevented by aminoguanidine, LTP measuring 203±12% and 143±11% at peak and 60 min post-HFS in the presence of Aβ and aminoguanidine. These values were not significantly different from control values (n=5, $p>0.05$, Fig 3.22), but were significantly increased from the values in Aβ alone ($p<0.005$, n=5, F value = 67.4, $p<0.001$).

We then investigated the effects of selective iNOS inhibitor, 1400W. 1400W is a selective iNOS inhibitor, Kd =7 nM, with a 5000- and 200-fold greater potency against iNOS relative to eNOS and nNOS (Garvey, 1997). In control slices, 1400W (2 μM) did not alter control LTP induction, which measured 223±17% and 167±5%, at peak and 60 min post-HFS respectively (significant LTP, $p<0.001$, n=5, Fig 3.23). A two-way ANOVA showed no significant difference between LTP in control and in 1400W, F value = 1.4, $p=0.246$). However, the Aβ-mediated inhibition of LTP induction was prevented by 1400W, LTP measuring 199±8% and 150±8% at peak and 60 min post-HFS in the presence of Aβ

and 1400W. These values were not significantly different from control values ($n=5$, $p>0.05$, Fig 3.23), but significantly increased from the values in Aβ alone ($p<0.005$, $n=5$, F value $=21.6$, $p<0.002$). The results of these experiments with aminoguanidine and 1400W further support a role for iNOS in Aβ-mediated inhibition of LTP induction.

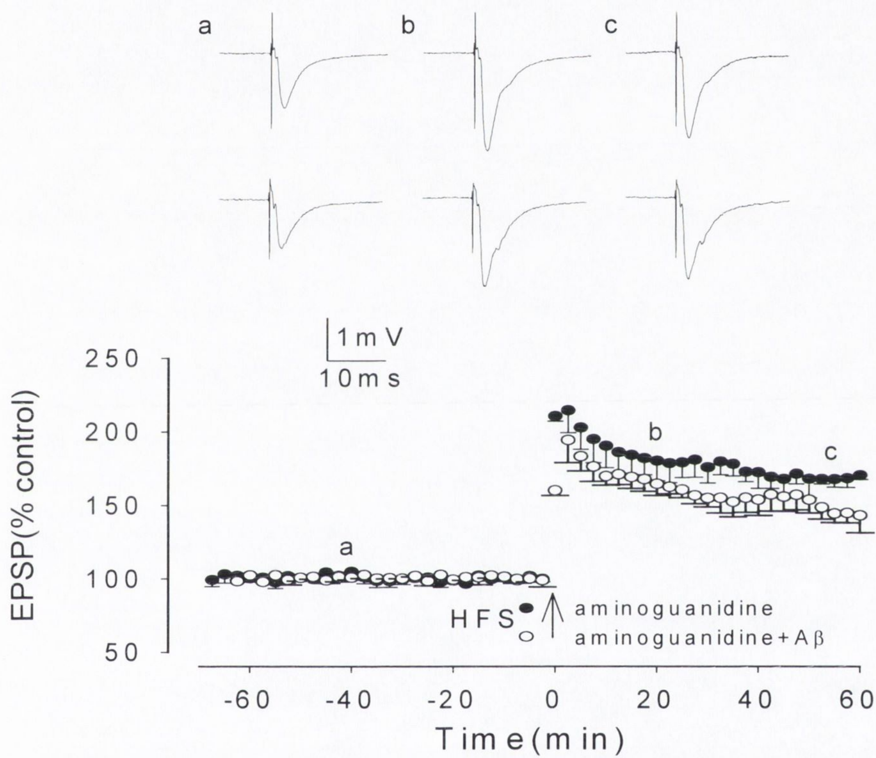

Fig.3.22 Induction of LTP in the presence of aminoguanidine (filled circles, n=5), not significantly reduced from control, and in the presence of aminoguanidine plus Aβ (open circles, n=5). Aminoguanidine prevented the Aβ-mediated inhibition of LTP

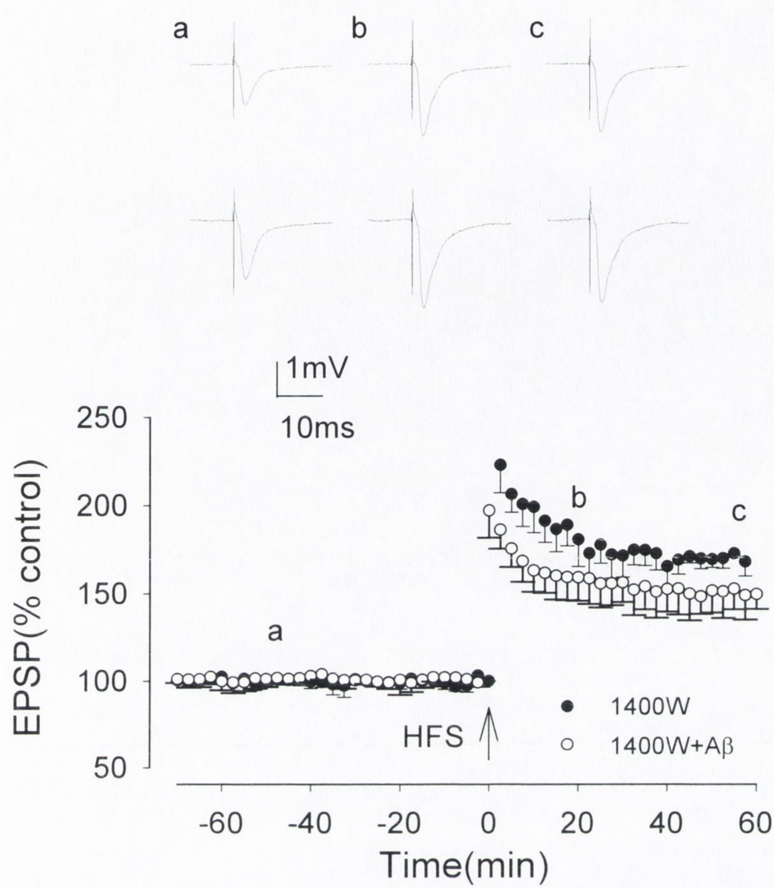

Fig.3.23 Induction of LTP in the presence of 1400W (filled circles, n=5), not significantly reduced from control, and in the presence of 1400W plus Aβ (open circles, n=5). 1400W prevented the Aβ-mediated inhibition of LTP induction.

3.8 Effect of superoxide on Aβ – induced LTP impairment

3.8.1 Aβ-mediated inhibition of LTP induction is prevented by a combination of superoxide dismutase and catalase.

In order to investigate the role of the reactive oxygen species (ROS) superoxide, we examined the effect of the superoxide scavenger superoxide dismutase (SOD) on Aβ-mediated inhibition of LTP induction. SOD is a specific enzyme that only catalyses the removal of superoxide (Klann et al, 1998). In confirmation of previous studies (Klann et al, 1998), SOD applied alone inhibited LTP induction. Thus in the presence of SOD (100 units/ml, LTP measured 160±17% and 116±5% at peak and 60 min post-HFS ($p<0.005$, $n=5$, $F=80.6$, $p<0.001$, Fig 3.24). Such inhibition of LTP induction by SOD is most likely to be caused by hydrogen peroxide production, as SOD converts superoxide to oxygen and hydrogen peroxide, and the latter species is known inhibit LTP induction (Kamsler and Segal 2003). In order to overcome these effects of hydrogen peroxide on LTP induction, the effects of Aβ on LTP induction were examined in the presence of SOD plus catalase. Catalase is a scavenger of hydrogen peroxide, catalysing its conversion to oxygen and water. Catalase alone (260 units/ml) did not alter LTP induction, which measured 203±11% and 169±7% at peak and at 20 min and 60 min post-HFS ($n=5$, $p>0.05$, Fig 3.24). However, catalase prevented the inhibitory effects of SOD on LTP induction, LTP measuring 197±12% and 142±6% at peak and 60 min post-HFS in catalase plus SOD, values significantly increased from those in SOD alone ($p<0.005$, $n=5$), although lower than control ($p<0.005$, $n=5$).

The combination of SOD plus catalase was found to prevent the Aβ-mediated inhibition of LTP induction, LTP measuring 194±20% and

131±10% at peak and 60 min post-HFS respectively in the presence of Aβ plus SOD plus catalase. These values were significantly increased from the values in Aβ alone ($p<0.005$, n=5, F=59.4, $p<0.001$, Fig 3.25), although lower than control values ($p<0.005$, n=5). These experiments demonstrate a role for superoxide in the Aβ-mediated inhibition of LTP induction.

3.8.2 Aβ-mediated inhibition of LTP induction is prevented by an inhibitor of NADPH oxidase

NADPH oxidase is a common source of superoxide in cells, with activated NADPH catalysing the transfer of one electron from NADPH to oxygen, giving rise to superoxide. Diphenyleneiodonium (DPI) is an inhibitor that has been used in many studies to prevent the production of superoxide by NADPH oxidase (Qin et al, 2002). DPI (50 μM), did not inhibit LTP induction, which measured 212±17% and 167±6% at peak and 60 min post-HFS respectively (n=5, $p>0.05$, F = 0.13, p=0.73, Fig 3.26). However, DPI partially prevented the Aβ-mediated block of LTP induction, LTP measuring 180±15% and 146±7% at peak and 60 min post-HFS respectively in the presence of DPI plus Aβ. These values were not significantly different from control values (n=5, $p>0.05$, Fig 3.26), but significantly increased from the values in Aβ alone ($p<0.005$, F=26.1, $p<0.001$).

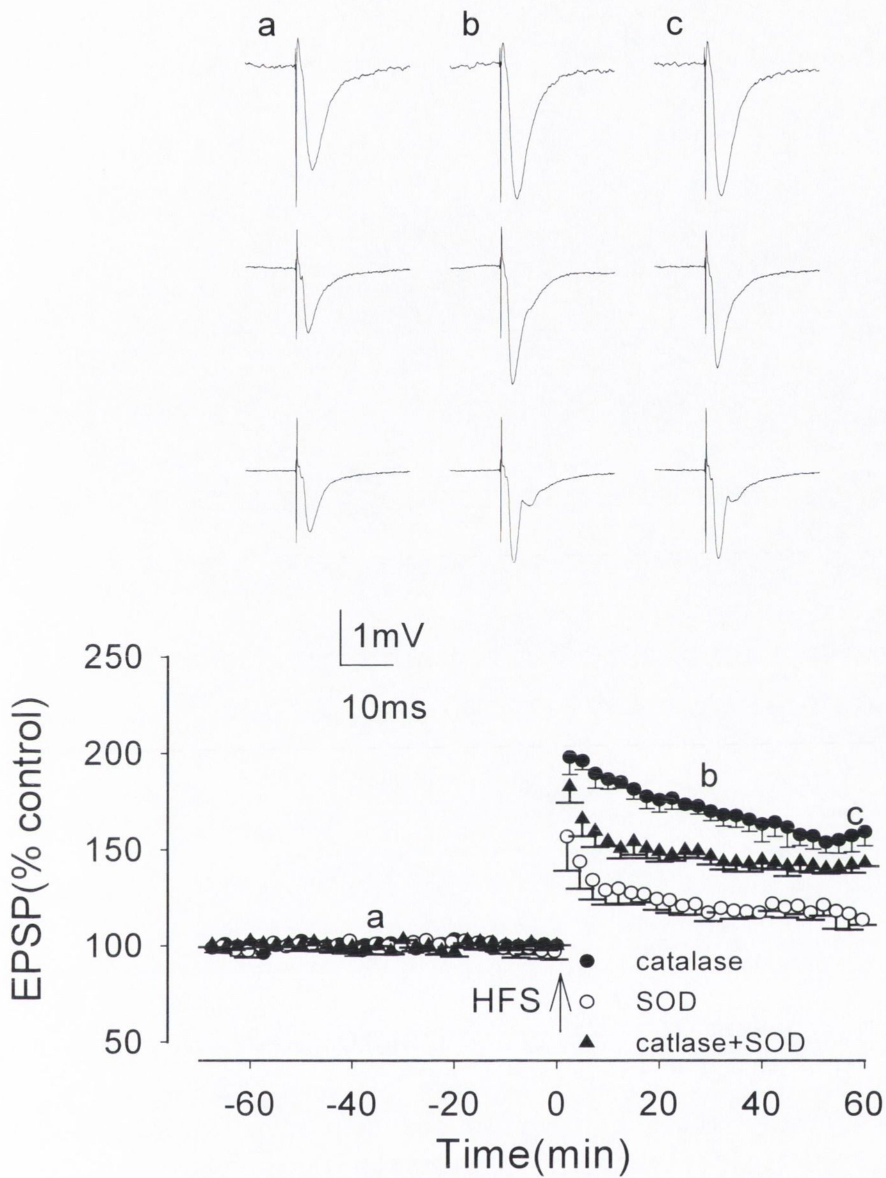

Fig.3.24 Induction of LTP in the presence of SOD (open circles, n=5), significantly reduced from control, in the presence of catalase (filled circles, n=5), not significantly reduced from control, and in the presence of SOD plus catalase(triangle, n=5), partially reduced from control.

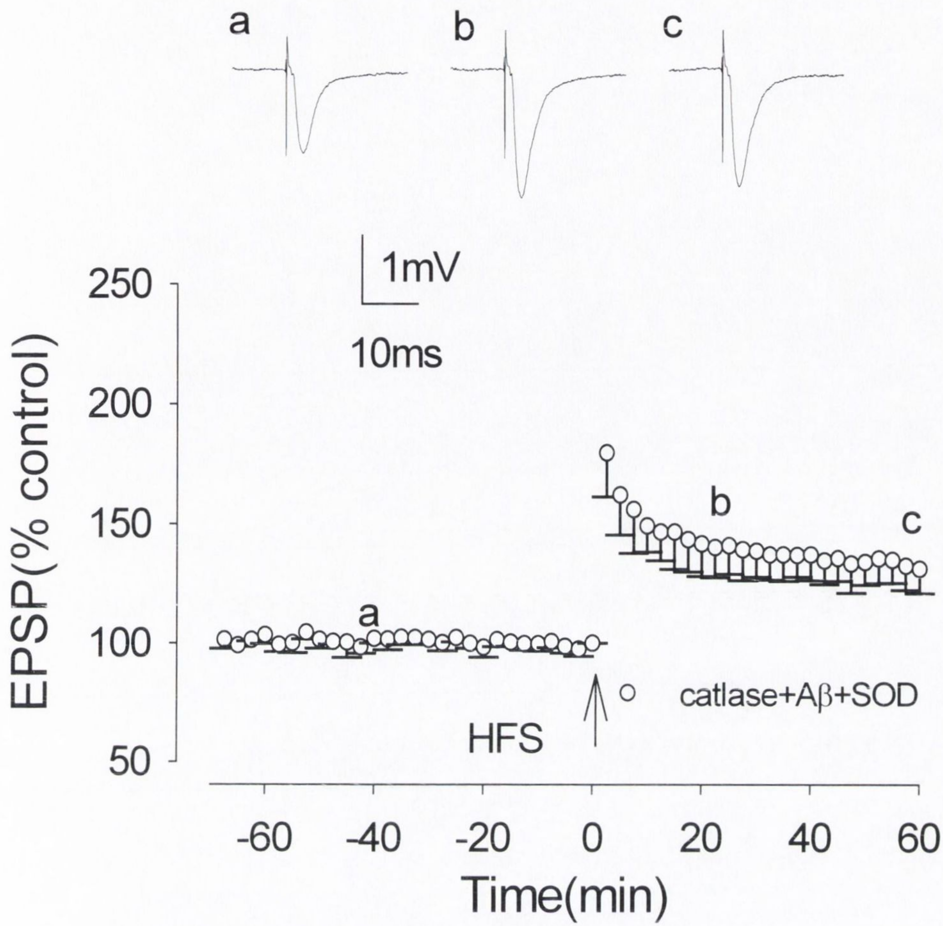

Fig.3.25 LTP induction in the presence of SOD plus catalase plus Aβ. The combination of SOD plus catalase prevented the Aβ-mediated inhibition of LTP induction (n=5).

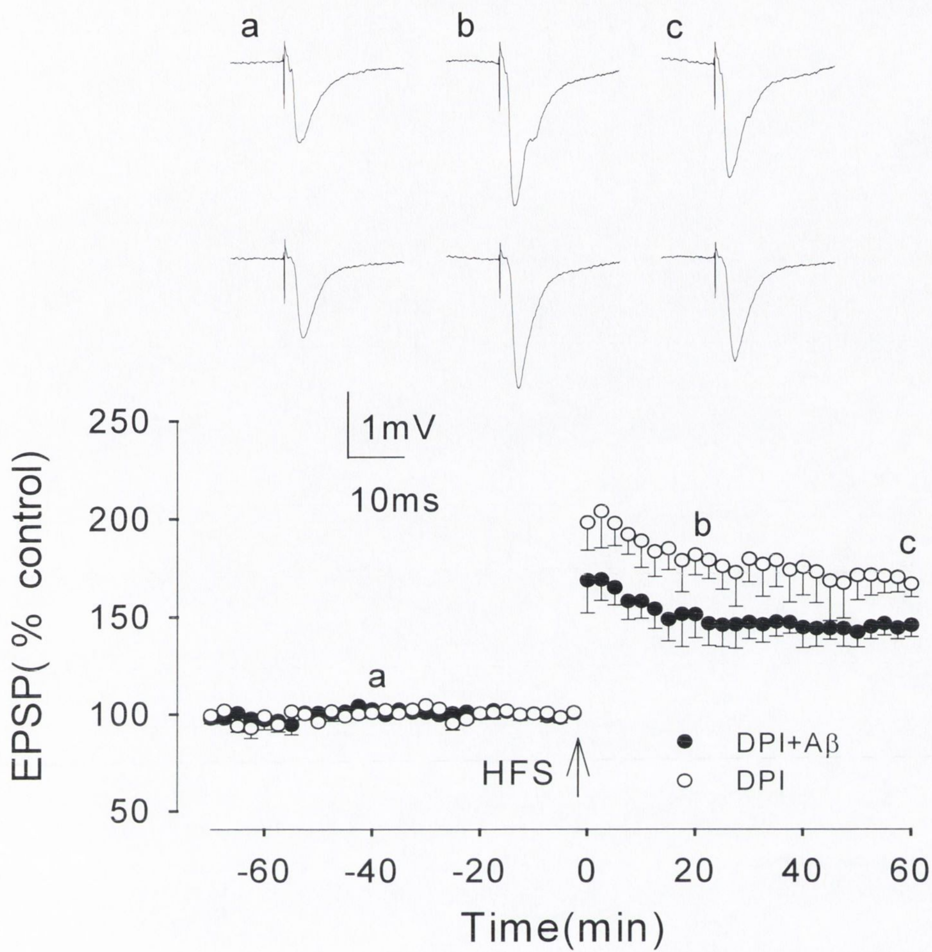

Fig.3.26 The Aβ-evoked inhibition of LTP induction is prevented by the NADPH oxidase inhibitor, diphenyleneiodonium (DPI) in rat slices. The graph shows that DPI partially prevents the Aβ-mediated inhibition of LTP induction (n=5).

3.9 Effects of COX inhibition on Aβ-induced inhibition of LTP

Several groups reported the relationship between COX and Aβ. For example, In AD, inflammatory cells expressing COX-1 surround Aβ deposits (Hoozemans et al., 2001), while COX-2 accumulates in neurons (Ho et al., 2001; Hoozemans et al., 2001; Pasinetti and Aisen, 1998). The protective effects of NSAIDs have been ascribed to their anti-inflammatory properties, involving inhibition of either COX-1 or COX-2(McGeer, 2000; van Gool et al., 2003). To address the possibility of synaptic COX in determining the beneficial effects of NSAIDS on memory, we examined the involvement of COX-2 in the induction of LTP in rat hippocampal slices exposed to soluble Aβ. The selective COX-2 inhibitors MF tricyclic and NS398 prevented the inhibition of LTP by Aβ, LTP measuring 134±8% (n=5, P<0.01) and 143±3% (n=5, P<0.01) at 60min after HFS in the presence of Aβ plus MF tricyclic and NS-398 respectively (Fig 3.27 and Fig 3.28). In contrast, the selective COX-1 inhibitor piroxicam failed to restore LTP disrupted by Aβ, LTP measuring 105±7% in the presence of Aβ plus piroxicam (n=5, P>0.05) (Fig 3.29). Neither the COX-2 inhibitors MF tricyclic and NS398 or the COX-1 inhibitor piroxicam altered LTP induction in the absence of Aβ, LTP measuring 152±5%, 163±4% and 157±7% in the presence of MF tricyclic, NS398 and piroxicam respectively (n=5, P>0.05) (Fig 3.27-29). Thus the inhibition of synaptic COX-2 activity was sufficient to restore longlasting potentiation which had been disrupted by Aβ.

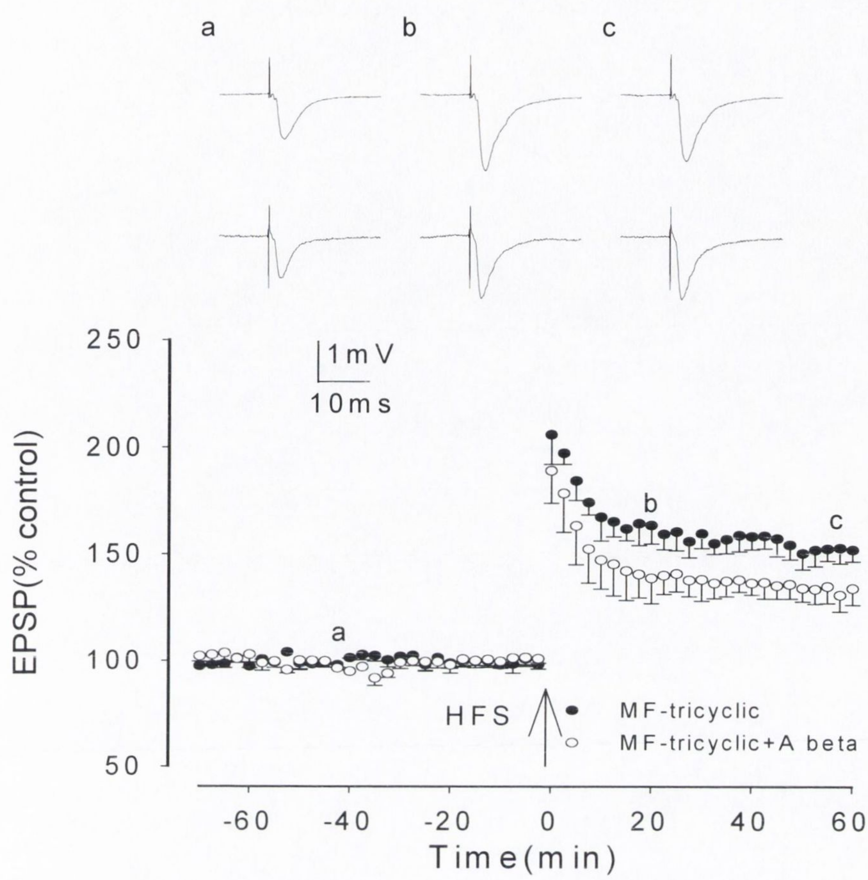

Fig.3.27 The COX-2 inhibitor MF tricyclic (3 μ M), applied 60 min prior to HFS, does not inhibit LTP induction (closed circles, n=5) but prevents the A β -mediated inhibition of LTP induction (open circles, n=5).

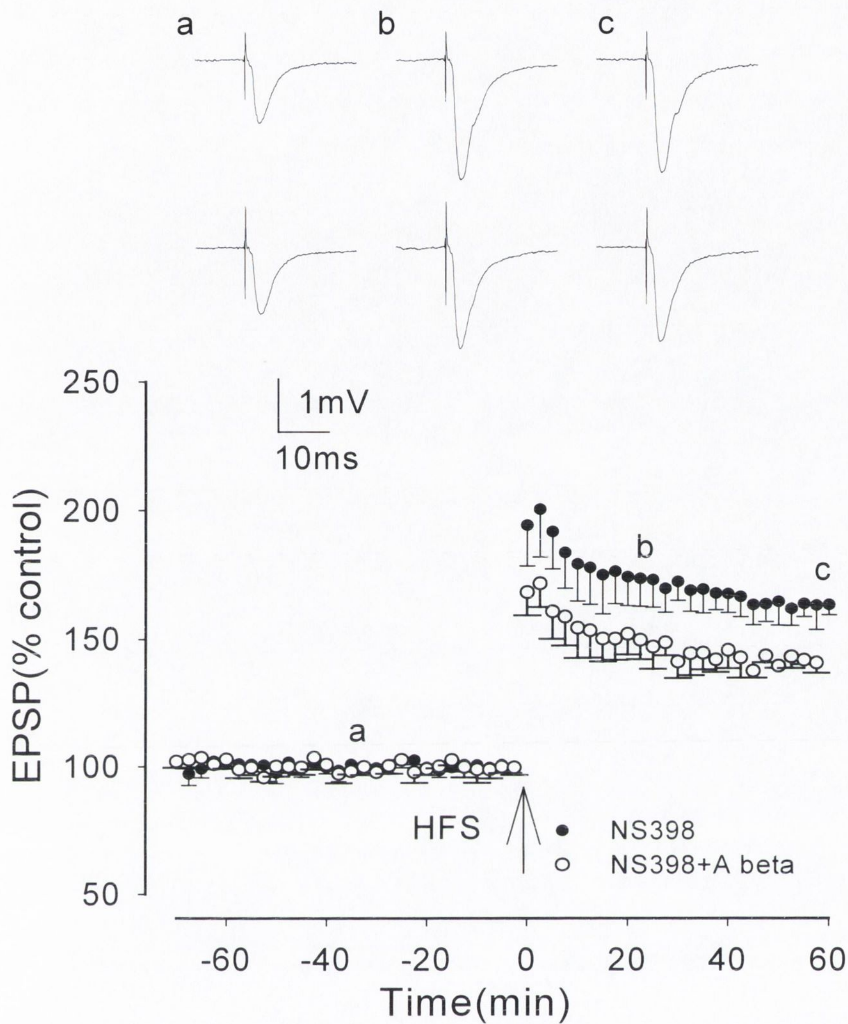

Fig.3.28 The COX-2 inhibitor NS-398 (2 μM), applied 60 min prior to HFS, does not inhibit LTP induction (closed circles, n=5) but prevents the Aβ-mediated inhibition of LTP induction (open circles, n=5).

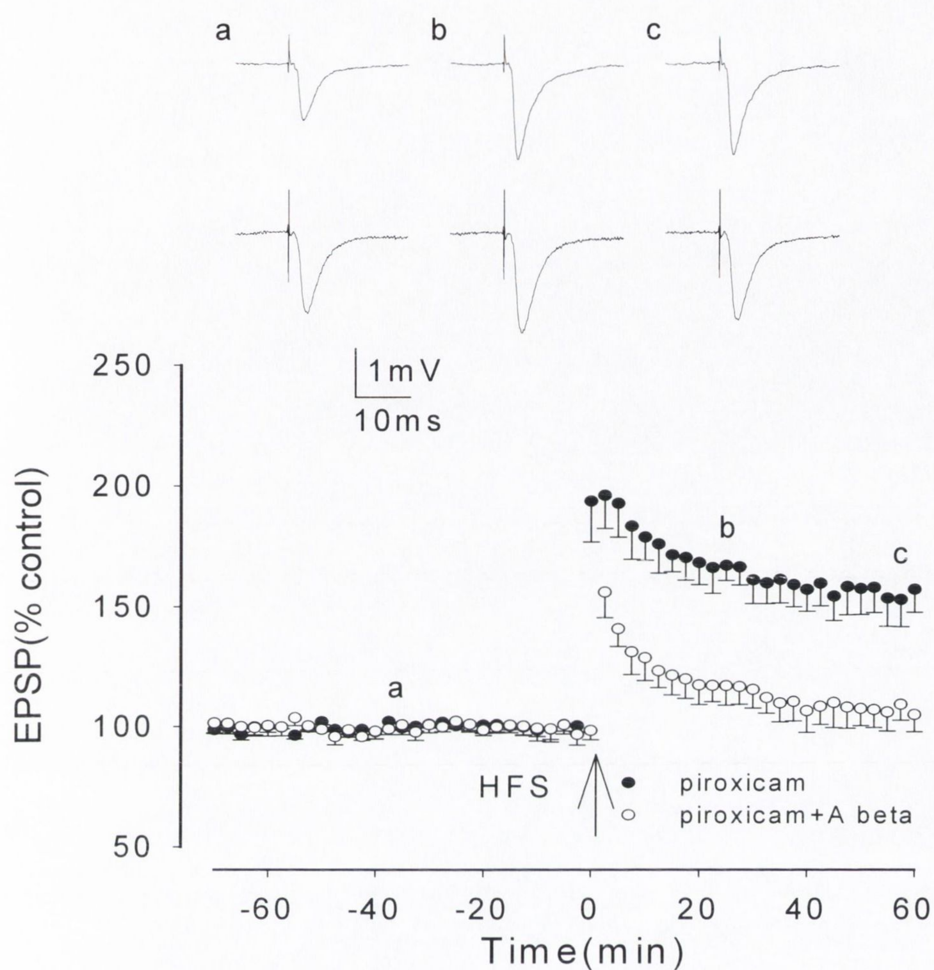

Fig.3.29 The COX-1 inhibitor piroxicam (10 μ M), applied 60 min prior to HFS, does not inhibit LTP induction (closed circles, n=5) and also does not prevents the A β - mediated inhibition of LTP induction (open circles, n=5).

3. 10 The inhibition of LTP by Aβ involves activation of mGluR5, but not the α7 nicotinic receptor.

In order to identify whether the inhibitory action of Aβ on LTP induction is mediated via activation of the specific transmitter receptors mGluR5 or α7 nAChR, synthetic Aβ was applied in the presence of selective antagonists of these receptors. LY341495 has been shown to be a selective group I/group II mGluR antagonist (Fitzjohn et al, 1998; Kingston et al, 1998). This antagonist does not inhibit the induction of LTP in CA1 (Fitzjohn et al, 1998) or, in previous experiments from this laboratory, in the medial perforant path of the dentate gyrus (Rush et al, 2002). LY341495 was found to prevent the Aβ-mediated inhibition of LTP induction. In the presence of LY341495 (10 μM), and Aβ, LTP measured 192±14%, 149±5% and 147±3%, at peak, 20 min and 60 min post-HFS, values significantly increased from the values in Aβ alone ($n=5$, $p<0.05$) but not significantly different from control LTP ($p>0.05$) (Fig 3.30). The selective mGluR5 antagonist MPEP (5 μM) (Gasparini et al, 1999) also prevented the Aβ-mediated inhibition of LTP induction. In the presence of MPEP, LTP measured 192±15%, 155±4% and 141±9%, at peak, 20 min and 60 min post-HFS, values significantly increased from LTP in Aβ alone ($n=5$, $p>0.01$), but not significantly different from control LTP ($p<0.05$) (Fig 3.31).

In view of reports that Aβ has been found to bind to and activate α7 nAChR at pM concentrations (Dinerly et al, 2001; 2002), the action of the selective α7 nAChR antagonist methylcaconatine (MLA) was investigated on the ability of Aβ to inhibit LTP induction. MLA (1 μM)

did not prevent the Aβ-mediated inhibition of LTP, LTP measuring 154±11%, 113±6% and 105±5% at peak, 20 min and 60 min post-HFS, values not significantly different from those in Aβ alone (n=5, $p>0.05$) (Fig 3.32). on the ability of Aβ to inhibit LTP induction. MLA (1 μM) did not prevent the Aβ-mediated inhibition of LTP, LTP measuring 154±11%, 113±6% and 105±5% at peak, 20 min and 60 min post-HFS, values not significantly different from those in Aβ alone (n=5, $p>0.05$) (Fig 3.32).

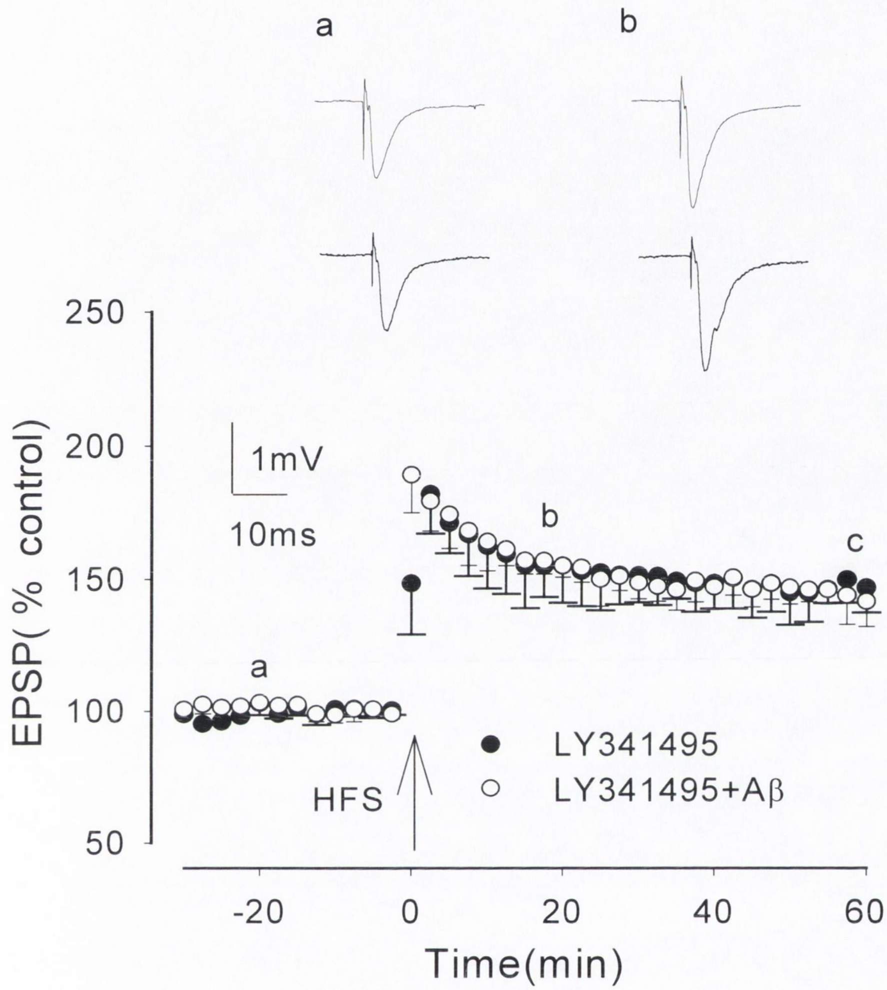

Fig 3.30 The induction of LTP in the presence of 500 nM synthetic Aβ plus the group I/II antagonist LY341495(open circles, n=5), not significantly reduced from control (closed circles, n=4).

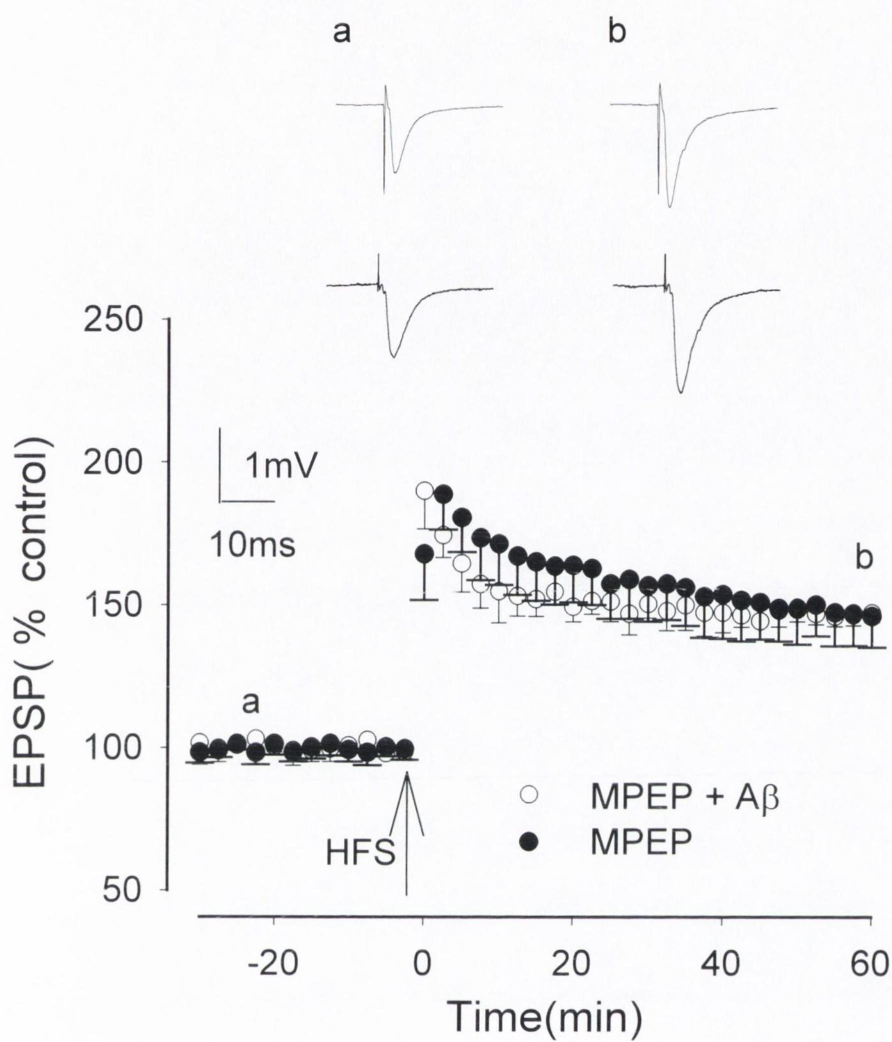

Fig 3.31 The induction of LTP in the presence of 500 nM synthetic Aβ plus the mGluR5 antagonist MPEP(open circles, n=5), not significantly reduced from control (closed circles, n=5).

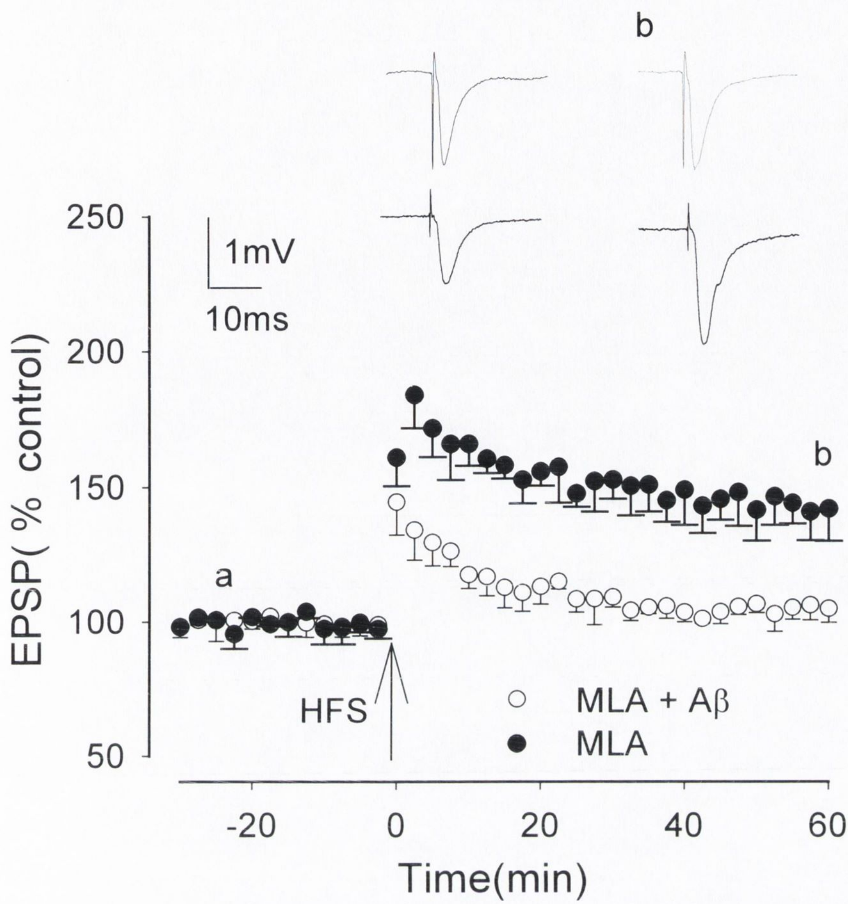

Fig 3.32 The induction of LTP in the presence of the α7 nAChR antagonist, methylcaconatine (MLA) and 500 nM Aβ (open circles, n=5), significantly reduced from control (closed circles, n=4).

4. Discussion

4.1 Effect of Aβ on basal synaptic transmission and synaptic plasticity

The lack of effect of Aβ on membrane potential, input resistance, and baseline EPSPs demonstrates that Aβ does not cause a acute deleterious effect on cell viability or on inhibition of AMPA receptors contributing to baseline EPSPs. Although a longer perfusion with high concentrations of Aβ1–42 cause a long-lasting depression of baseline synaptic transmission in the hippocampus, consistent with the previous research (Cullen et al., 1997; Hartell and Suh, 2000). Cullen et al. reported (1996) a delayed (>5 hr) decline in synaptic transmission lasting at least 48 hr after a single intracerebroventricular injection of a low dose of Ab1–40 , and the reduction is NMDA receptor dependent. In the case of CT, but not Ab1–42, the depression was blocked by a nitric oxide synthase inhibitor and was associated with an increase in intracellular Ca2+ levels in cerebellar Purkinje cells (Hartell and Suh, 2000). This was proposed to be caused by the formation or opening of relatively nonselective cation channels in the plasma membrane. Such reductions in synaptic transmission are putative substrates for the reductions in cerebral activity of patients with preclinical Alzheimer's disease (Rapoport, 2000).

We demonstrates in present study that a low concentration of Aβ inhibits NMDAR-dependent LTP induction, confirming previous studies in CA1 *in vivo* and dentate gyrus *in vitro* (Cullen et al., 1997) and several

other studies in CA1 and dentate gyrus *in vitro* (Lambert et al., 1998; Itoh et al., 1999; Chen et al., 2000; Stephan et al., 2001; Vitolo et al., 2002; Raymond et al., 2003). The inhibitory effect of A β was found to be confined to a specific plasticity, that of the induction of NMDAR-dependent LTP. Thus A β did not inhibit the induction of NMDAR-independent LTP or of LTD, the latter observations in agreement with Wang et al. (2002) and Raymond et al. (2003). Such a selective action of Aβ on induction of plasticity suggests that Aβ inhibits a specific process essential to induction of NMDAR-dependent LTP. It is unlikely that Aβ results in inhibition of LTP via a block of activation of NMDAR, because Aβ produces only a very small inhibition of NMDAR-mediated EPSPs in this study and Raymond's, which is insufficient to reduce the induction of LTP (Raymond et al., 2003). However, NMDAR-dependent LTP is well known to involve activation of calcium/calmodulin kinase II (CaMKII) (for review, see Lisman et al., 2002) in contrast to that of NMDAR-independent LTP, which is independent of the activation of CaMKII (Cavus and Teyler, 1996). This suggests the possibility that receptor binding of Aβ may result in a block of the activation of CaMKII.

4.2 Effect of human cell-derived Aβ on NMDAR-independent LTP

In agreement with our previous report that of inhibition of LTP in CA1 *in vivo* (Walsh et al, 2002), we found that cell-derived, naturally secreted Aβ inhibits LTP induction *in vitro*. When solubilized in aqueous buffers, $A\beta_{1-42}$ assembles into a variety of structures, including low n-oligomers, ADDLs, protofibrils and fibrils (Walsh et al, 1997, Lambert et al, 1998; Bitan et al, 2002). The solutions of synthetic Aβ

used in this study contain a mixture of these different assemblies. In contrast, the Aβ present in the cell-derived 7PA2 CM are free of fibrils and protofibrils, and contained only Aβ monomers and soluble low n-oligomers (Walsh et al. 2002). The finding that cell-derived Aβ inhibits LTP *in vitro* confirms that soluble assemblies of Aβ can inhibit LTP. Cell-derived naturally secreted Aβ was much more potent than our synthetic Aβ preparation at inhibiting LTP induction. Thus the threshold inhibitory concentration for cell-derived Aβ was calculated to be ~0.7 nM, and that of synthetic Aβ between 100 nM and 200 nM (the values of the synthetic Aβ would be one-third lower than that stated if all batches of Aβ were identical to that determined – see Methods). The large difference in potency between cell derived and synthetic Aβ may be explained by only a very low concentration of biologically active oligomers of Aβ being present in the synthetic Aβ solution.

It is of interest that the very early phase LTP, including initial peak amplitude, was inhibited by cell-derived Aβ in the present *in vitro* studies, as our previous studies *in vivo* had shown that cell-derived Aβ only inhibited LTP beginning ~1 hr post HFS. This difference between the *in vitro* and *in vivo* studies may due to the longer period of pre-exposure to Aβ prior to HFS *in vitro* (60 min) compared with *in vivo* (10 min). Other possible explanations are a slower diffusion time of the Aβ after *in vivo* cannula injection compared with *in vitro* perfusion of Aβ, or a concentration difference since the Aβ injected *in vivo* is diluted by the cerebro-spinal fluid.

4.3 Effect of kinases inhibitors on Aβ – induced impairment LTP

Few studies have explored the mechanisms underlying the Aβ-mediated inhibition of LTP, although recently one study found that Aβ-mediated inhibition of LTP was reversed by rolipram and forskolin, drugs that enhance cAMP signalling (Vitolo et al, 2002). In the current study, we provide novel evidence that the Aβ-evoked inhibition of LTP is mediated via activation of the kinases JNK, Cdk5 and p38 MAPK. There is previous evidence for the involvement of JNK in the neuropathology of AD. JNK activation has been described around amyloid deposits in AD brains (Anderson et al, 1994; 1996) Shoji et al, 2000; Zhu et al, 2001a), and synthetic Aβ activated the JNK pathway in various neuronal systems (Bozyzcko-Coyne et al, 2001; Morishima et al, 2001; Troy et al, 2001). Although Aβ increases expression of the stress-activated gene transcription factor c-Jun (Anderson et al, 1994; Estus et al, 1997), such action is likely to be too slow to account for the rapid inhibition of LTP by Aβ observed in the present studies. Rather, a local synaptic action is more likely to be responsible for the effect of JNK inhibitors on the block of LTP. JNK activation is known to have a local cytoplasmic action leading to inhibition of dendritic growth (Coffey et al, 2000; Savage et al, 2002), and it is possible that inhibition of LTP by Aβ-mediated activation of JNK is an initial stage preceding such inhibition of dendritic growth.

The present evidence for involvement of p38 MAP kinase in the Aβ-mediated inhibition of LTP parallels previous studies showing an increase in p38 MAP kinase activity effected by Aβ in cultured cells and also an increase in MKK6, an upstream activator of p38, in susceptible neurons in AD brains (McDonald et al, 1998; Pyo et al, 1998). Activation of JNK and p38 MAPK are likely to result in the inhibition of LTP via inflammatory pathways. A growing body of evidence suggests that Aβ-

mediated neurotoxicity involves the production of inflammatory cytokines such as tumor necrosis factor (TNF), and also free radicals and reactive oxygen species (Akama et al, 2000). In this regard, activation of JNK and p38 MAPK are known to have a pivotal role in TNF signalling and cell death (Paul et al, 1997). The inhibition of LTP could be very early indicator of the activation of inflammatory mediators. The Aβ-mediated block of LTP was not prevented by the p42/44 MAP kinase inhibitor UO126, demonstrating a lack of involvement of p42/44 MAP kinases. In fact, p42/44 MAP kinase is known to be required for the induction, rather than inhibition, of LTP, and to be involved in cell survival rather than cell death (Sweatt, 2001).

The present finding that the Cdk5 inhibitors butyrolactone and roscovitine prevent the Aβ-mediated inhibition of LTP parallels recent studies showing that Cdk5 inhibitors prevent Aβ-induced neurotoxicity (Alvarez et al, 1999; Milton et al, 2001). Cdk5 has been postulated to have a major role in AD and Aβ-induced neurodegeneration (Ahlijanian et al, 2000; Patrick et al, 1999), with Aβ causing mislocalization and deregulation of Cdk5 by increasing production of its pathogenic activator p25 (Patrick et al. 1999, Lee et al, 2000, Twon et al, 2002.

4.4 Inhibition of microglia activation prevents Aβ inhibition of LTP

Accumulating experimental evidence suggests a causal link among A β, oxidative stress, and AD. The present studies provide similar links among A β, oxidative stress, and the inhibition of LTP. We provide evidence that the A β-mediated inhibition of LTP induction involves activation of microglia, the principle immune effector cell in the brain.

This conclusion is based on the results of our studies with minocycline, a tetracycline inhibitor of microglial activation, which was found to prevent the action of Aβ in blocking induction of LTP. Although minocycline has not been reported previously to prevent the microglial activation produced by A β, it has marked *in vivo* neuroprotective properties in experimental models of neurodegeneration, including cerebral ischemia, traumatic brain injury, Huntington's disease, Parkinson's disease and amyotrophic lateral sclerosis (Yrjanheikki et al., 1998; Chen et al., 2000; Du et al., 2001; Sanchez Mejia et al., 2001; Zhu et al., 2002). Minocycline also has been found to be neuroprotective in a variety of *in vitro* models of cell death, with a rapid action similar to that observed in the present studies on LTP. For example, 30 min preapplication of minocycline to spinal cord cultures inhibited the rapid (10 min) excitotoxin-induced effects of excitatory amino acids (Tikka et al., 2001). Zhu et al. (2002) also showed a rapid protective effect of minocycline against NMDAR-mediated cell death in acute studies in primary cortical neurons. The mechanisms of action of minocycline underlying its neuroprotective effect include inhibition of cytochrome *c* release, caspases, and p38 MAPK (Yrjanheikki et al., 1998; Tikka et al., 2001; Zhu et al., 2002). Interestingly, in a recent study we showed that activation of p38 MAPK is required for Aβ-mediated inhibition of LTP induction (Wang et al., 2004).

The finding of an involvement of microglia in A β-mediated inhibition of LTP supports previous evidence for an involvement of microglia in AD. The pathology of AD has been well documented to involve a chronic inflammatory reaction driven by activated microglia (Griffin et al., 1998; Akiyama et al., 2000); abundant reactive microglia surround β-amyloid plaques in the AD brain (McGeer and McGeer, 1995;

Cotman and Su, 1996), and treatment with anti-inflammatory agents has been correlated with a large reduction in plaque-associated reactive microglia and a decrease in severity of AD (MacKenzie and Munoz, 1998). In addition, A β causes activation of microglia cells in culture (Tan et al., 1999; Akiyama et al., 2000; Combs et al., 2000; Bamberger et al., 2003), and activated microglial cells have been shown to be the link between A β deposition and neuronal death (Eikelenboom et al., 1994; McGeer and McGeer, 1995).

4.5 Effect of NO and superoxide on Aβ – induced LTP impairment

Evidence for a requirement for activation of iNOS in the A β-mediated inhibition of LTP induction was found in the present study via the use of iNOS mutant mice and two selective inhibitors of iNOS, aminoguanidine and 1400W. Increased levels of iNOS have been found in cells surrounding amyloid plaques (Wallace et al., 1997), and A β can stimulate iNOS in microglia *in vitro* and *in vivo* (Akama et al., 1998; Akama and Van Eldik, 2000). Stimulation of iNOS catalyzes the oxidation of L-arginine to L-citrulline and nitric oxide. Although nitric oxide is a free radical, the main cytotoxic action of NO is most likely attributable to production of peroxynitrite.

Evidence was presented in the present study for the involvement of superoxide in the A β-mediated inhibition of LTP induction. Thus a combination of the superoxide scavenger SOD and the hydrogen peroxide scavenger catalase prevented the Aβ-mediated inhibition of LTP induction. Moreover, inhibition of NADPH oxidase by DPI was found to prevent the Aβ-mediated inhibition of LTP induction. Activation of NADPH oxidase

has been shown previously to be a major source of superoxide production involved in Aβ-induced toxicity (Bianca et al., 1999), and the Aβ-induced production of superoxide was blocked by the NADPH oxidase inhibitor DPI (Qin et al., 2002). We postulate that the active cytotoxic species responsible for the A β-mediated block of LTP is most likely to be peroxynitrite, formed by the combination of NO and superoxide (Lipton et al., 1993). The reaction between superoxide and nitric oxide to produce peroxynitrite is very rapid and out-competes the major scavenging pathways for nitric oxide and superoxide. Peroxynitrite is a highly reactive strong oxidant and nitrating agent and is known to cause neuronal death in cultured cells (Lin et al., 1998).

How might an oxidative/inflammatory-like block of LTP by A β help explain the pathophysiology and symptoms of AD? Several theories of AD have implicated Aβ-triggered oxidative/inflammatory-like effects in the development of the disease (for review, see McGeer and McGeer, 2003). Moreover, previous studies have presented strong evidence that synapses are the initial target in AD (for review, see Selkoe, 2002). The hippocampus is especially vulnerable in AD, and early debilitating symptoms usually include significant deficits in the performance of hippocampus-dependent cognitive tasks. Given the evidence that LTP underlies hippocampus-dependent learning and memory (Martin and Morris, 2002), the present findings of a block of LTP by A β provide a plausible mechanism for the impairment of cognitive functions in AD, especially early in the disease process. In addition, because plasticity at synapses is important for their survival (Toni et al., 1999; Hering and Sheng, 2001), any persistent disruption of LTP induction mechanisms may provide a very sensitive predictor of synaptic vulnerability to degeneration.

4.6 Effect of COX inhibition on Aβ-induced inhibition of LTP

Results in present study indicate that the inhibition of LTP by Aβ is dependent on the activation of COX-2, but not COX-1. In a recent study, we also showed COX-2 inhibition restored memory function without reducing either Aβ or TNFα, IL-1β in a transgenic model of AD (Westerman et al., in press). Previous studies have shown that a role for COX-2 in synaptic plasticity since NS-398 was found to inhibit LTP in the dentate gyrus of 2-3 months male c-57 mice(chen et al., 2002). This effect was reversed by PGE_2, the major oxidation product of arachidonic acid (AA). AA has previously been implicated in LTP as a potential retrograde messenger (Williams et al 1989), and is thought to act by altering the binding of glutamate to the AMPA receptors (Massicotte at al., 1991). However we didn't find the inhibition of COX-2 inhibitor NS-398 or MF-tricyclic in present study. The difference may in part due to the exposure time and concentration since a recent study has shown that the COX-2 mediated inhibition of LTP that is time- and concentration-dependent (Murray et al., 2003) Murray et al. found that LTP was inhibited only after a longer 2 hours exposure to NS398.

How might COX-2 inhibition lead to improved synaptic plasticity and memory? Although mice with transgenes constitutively increasing neuronal COX-2 develop memory deficits (Andreasson et al., 2001), we recently found no increase in COX-2 in Tg2576 mice, despite observing beneficial effects of COX-2 inhibitors on memory (Westerman et al., in press). The lack of change in COX-2 is consistent with other reports in aged Tg2576 mice showing no increase in PGE2, and COX-2, and no increase in COX-2 mRNA in microarray analyses (Dickey et al., 2003;

Quinn et al., 2003). The COX-2 inhibitor inhibited COX-2 stimulated by Aβ but spared basal COX-2, since non-transgenic mice were not significantly impaired. These observations suggest that abnormal stimulation of COX-2, independently of increased levels of expression, is sufficient to disrupt brain function, and therefore inhibiting COX-2 restores function.

There are several possible explanations for how Aβ -mediated inhibition of plasticity might be dependent upon COX-2 in the absence of up-regulation of the enzyme or increased levels of PGE2. One possible explanation would involve a change in the cellular locus of COX-2 or its products in response to Aβ without an overall increase in COX-2 activity. Another possible explanation would be an increase in COX-2 or PGE2 turnover, without a dramatic change in levels of COX-2 or PGE2. A third possibility would be that COX-2 plays a permissive role in the inhibition of plasticity and memory by Aβ that does not need to be accompanied by over-activation of the enzyme once other pathways are altered by Aβ.

4.7 Effects of mGluR and α7nAChR inhibition on Aβ-induced inhibition of LTP

The group I/II mGluR antagonist LY341495 and the mGluR5 antagonist MPEP were found to prevent the Aβ-mediated inhibition of LTP, thus demonstrating the involvement of mGluR5 in the process. Selective antagonism of mGluR5 with MPEP has previously been shown to be neuroprotective against Aβ toxicity in cortical cultures (Bruno et al, 2000), emphasising the parallels between Aβ-mediated inhibition of LTP

and Aβ-mediated neurotoxicity. In addition, the membrane depolarisation evoked by relatively high concentrations of synthetic $A\beta_{1-42}$ was reported to involve activation of group I mGluRs (Blanchard et al, 2002). The involvement of group I mGluRs in the Aβ-mediated inhibition of LTP found in the present study could be due to direct binding and activation of the mGluRs by Aβ. However, in the absence of direct evidence for such binding, we speculate that it is more likely that the mGluR5 are activated by HFS. HFS will strongly activate mGluR5 as such receptors are located perisynaptically and will be particularly well stimulated by spillover of glutamate during HFS. Aβ is likely to be activating one or more surface receptors, as it is not readily taken up into cells of the dentate gyrus in slices even after several days in culture (Bi et al, 2002). Thus it is most probable that Aβ is stimulating JNK, Cdk5 and p38 MAPK via the activation of such surface receptors. The requirement for activation of group I mGluR in the Aβ-mediated inhibition of LTP may be due to the necessity for additional stimulation of kinases. Thus the activation of group I mGluRs is likely to stimulate Cdk5 and p38 MAPK, as recent work has shown that a group I mGluR agonist stimulates Cdk5, an action blocked by butyrolactone (Liu et al, 2001) and p38 MAP kinase (Bolshakov et al, 2000; Rush et al, 2002).

Antagonism of α7 nAChR did not prevent the Aβ block of LTP, demonstrating a lack of involvement of the α7 nAChR in Aβ-mediated block of LTP in our paradigm. Aβ has been found to bind to, and activate, α7 nAChR at pM concentrations (Dinerly et al, 2001a; 2001b). One possible reason for the lack of effect of blocking α7 nAChR on Aβ-mediated inhibition of LTP is that α7 nAChRs have been found to be

located only at high density on interneurons in the dentate gyrus, and only at very low density on granule cells (Dobelis et al, 2002).

4.8 Conclusion

The data demonstrate that A β causes inhibition of LTP induction by a series of events. We suggest that A β initially activates a target on microglia (and probably other glial cells or neurons), which could be a scavenger receptor (El Khoury et al., 1996), a receptor for advanced glycation end products (Yan et al., 1996), a receptor complex including a scavenger and integrin proteins (Bamberger et al., 2003), or the tumor necrosis factor type I receptor (TNFRI) (Li et al., 2004). The latter possibility is particularly intriguing because soluble A β, the species known to be active in inhibiting LTP, has been found to bind potently to TNFRI (Li et al., 2004). Aβ binding to rapidly initiates or promotes a stress cascade that eventually leads to disruption of neuronal NMDA receptor function/calcium homeostasis and inhibition of neuronal kinases that are essential for LTP induction We show that microglial activation leads to production of NO and superoxide, which then react to form peroxynitrite. Peroxynitrite probably results in the inhibition of LTP via oxidation/tyrosine nitration of a particular protein necessary for LTP induction. We also demonstrated an essential role for p38 and JNK activation in the A β-mediated inhibition of LTP (Wang et al., 2004). Activated p38 MAPK and JNK have been immunolocalized to microglia associated with amyloid plaques in the AD brain (Hensley et al., 1999), and they are well known to be activated by oxidative stress (Cobb, 1999) and to be linked to production of iNOS and superoxide. Thus we propose that the presence of excess levels of A β, especially soluble oligomeric

Aβ, activate p38 MAP kinase and JNK which in turn activate iNOS and NADPH to produce nitric oxide and superoxide free radicals, respectively. These radicals react to form the highly toxic peroxynitrite free radical which can overcome endogenous antioxidant defence mechanisms and cause dysregulation of LTP induction pathways at synapses, either at the level of the NMDA receptor or downstream signalling cascades(Fig 4.1).

The ability of Aβ-triggered oxidative stress/pro-inflammatory processes to potently inhibit LTP, a neurobiological model of the type of synaptic plasticity required for learning and memory, provides a plausible mechanism for impairment of cognition very early in AD. In addition, since plasticity at synapses is important for their survival, any persistent disruption of LTP induction mechanisms may provide a very sensitive predictor of synaptic vulnerability to degeneration, leading to synaptic loss, one of the hallmarks of clinical AD pathology.

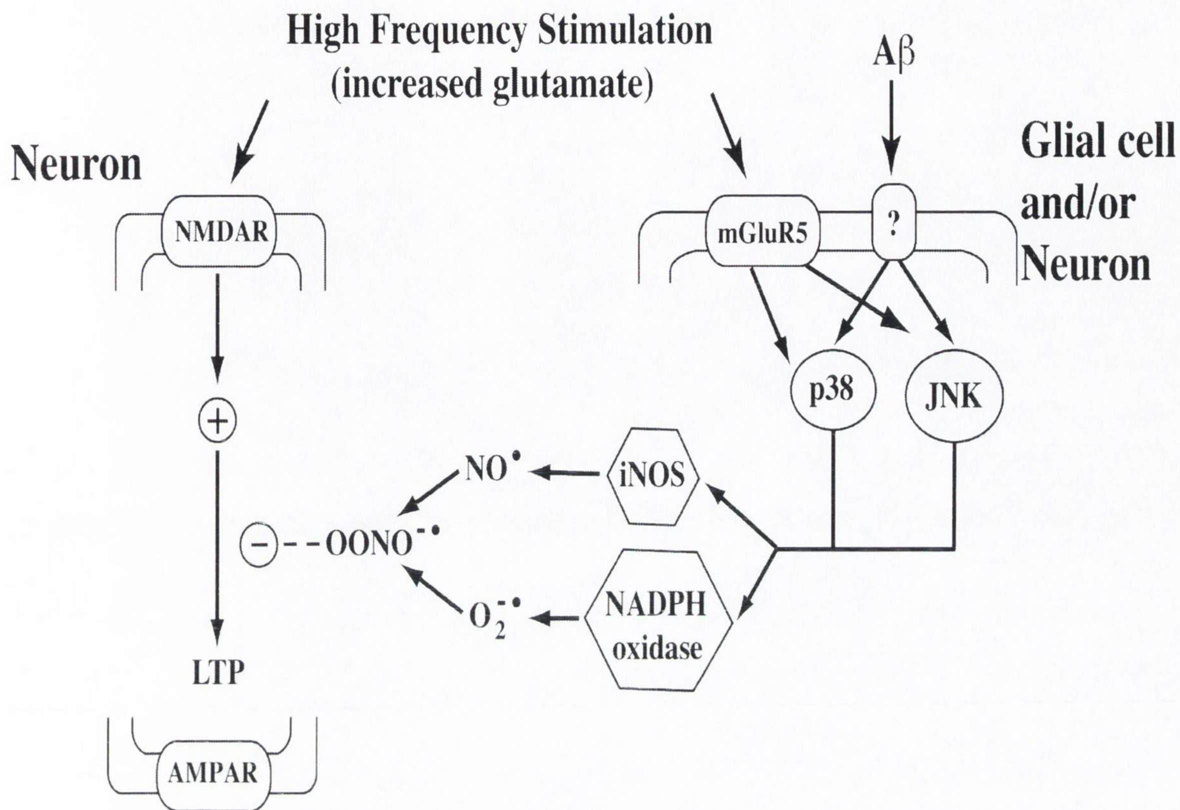

Fig. 4.1 Hypothetical schema of how oligomeric Aβ inhibits LTP induction.

5 Reference

Abe K and Saito H. (2000) Amyloid beta neurotoxicity not mediated by the mitogen-activated protein kinase cascade in cultured rat hippocampal and cortical neurons. Neurosci Lett. Sep 29;292(1):1-4.

Abraham WC and Goddard GV. (1983) Asymmetric relationship between homosynaptic long-term potentiation and heterosynaptic long-term depression. Nature, 305, 717–719.

Adams JP and Sweatt JD. Molecular psychology: roles for the ERK MAP kinase cascade in memory. Annu Rev Pharmacol Toxicol. 2002;42:135-63.

Ahlijanian MK, Barrezueta NX, Williams RD, Jakowski A, Kowsz KP, McCarthy S, Coskran T, Carlo A, Seymour PA, Burkhardt JE, Nelson RB, McNeish JD (2000) Hyperphosphorylated tau and neurofilament and cytoskeletal disruptions in mice overexpressing human p25, an activator of Cdk5. Proc Natl Acad Sci U S A. 97, 2910-5.

Ahn NG and Krebs EG. (1990) Evidence for an epidermal growth factor-stimulated protein kinase cascade in Swiss 3T3 cells. Activation of serine peptide kinase activity by myelin basic protein kinases in vitro. J Biol Chem. Jul 15;265(20):11495-501.

Aiba A, Chen C, Herrup K, Rosemund C, Stevens CF, and Tonegawa S (1994) Reduced hippocampal long-term potentiation and context-specific

deficit in associative learning in mGluR1 mutant mice. Cell. Oct 21;79(2):365-75.

Akama KT and Van Eldik LJV. (2000) β-amyloid stimulation of inducible nitric oxide synthase in astrocytes is IL-1□ and TNF-α dependent. J. Biol. Chem 275, 7918-7924.

Akama KT, Albanese C, Pestell RG and Van Eldik LJ (1998) Amyloid-peptide stimulates nitric oxide production in astrocytes through an NF-kB-dependent mechanism. Proc. Natl. Acad. Sci USA 95, 5795-5800.

Akama KT and Van Eldik LJ. (2000) Beta-amyloid stimulation of inducible nitric-oxide synthase in astrocytes is interleukin-1 beta- and tumor necrosis factor-alpha (TNFalpha)-dependent, and involves a TNFalpha receptor-associated factor and NFkappaB-inducing kinase-dependent signaling mechanism. J Biol Chem 275:7918.

Akhondzadeh S and Stone TW. (1995) Induction of a novel form of hippocampal long-term depression by muscimol: involvement of $GABA_A$ but not glutamate receptors. British Journal of Pharmacology, 115, 527–533.

Akhondzadeh S and Stone TW. (1996) Maintenance of muscimol-induced long-term depression by neurosteroids. Progress in Neuropsychopharmacology and Biological Psy chiatry, 20, 277–289.

Akhondzadeh S and Stone TW. (1996) Glutamate-independent long-term depression in rat hippocampus by activation of $GABA_A$ receptors. Life Science, 58, 1023–1030.

Akhondzadeh S and Stone TW. (1996) Muscimol-induced long-term depression in the hippocampus: lack of dependence on extracellular calcium. Neuroscience, 71, 581–588.

Akhondzadeh S and Stone TW. (1998) Potentiation of Mus-cimol-induced long-term depression by benzodiazepines and prevention or reversal by pregnenolone sulfate. Phar macological Research, 38, 441–448.

Akhondzadeh S and Stone TW. (1999) Prevention of muscimol-induced long-term depression by brain-derived neurotrophic factor. Progress in Neuropsychopharmacology and Biological Psychiatry, in press.

Akiyama H, Barger S, Barnum S, Bradt B, Bauer J, Cole GM, Cooper NR, Eikelenboom P, Emmerling M, Fiebich BL, Finch CE, Frautschy S, Griffin WS, Hampel H, Hull M, Landreth G, Lue L, Mrak R, Mackenzie IR, McGeer PL, O'Banion MK, Pachter J, Pasinetti G, Plata-Salaman C, Rogers J, Rydel R, Shen Y, Streit W, Strohmeyer R, Tooyoma I, Van Muiswinkel FL, Veerhuis R, Walker D, Webster S, Wegrzyniak B, Wenk G and Wyss-Coray T. (2000). Inflammation and Alzheimer's disease. Neurobiol. Aging 21, 383-421.

Alkondon M, Pereira EF, Barbosa CT and Albuquerque, EX. (1997) Neuronal nicotinic acetylcholine receptor activation modulates

gammaaminobutyric acid release from CA1 neurons of rat hippocampal slices. J. Pharmacol. Exp. Ther. 283, 1396–1411.

Alvarez A, Munoz JP, and Maccioni RB. (2001) A Cdk5-p35 stable complex is involved in the beta-amyloid-induced deregulation of Cdk5 activity in hippocampal neurons.Exp Cell Res. Apr 1;264(2):266-74.

Alvarez A , Toro R, Caceres A and Maccioni RB (1999) Inhibition of tau phosphorylating protein kinase Cdk5 prevents β-amyloid-induced cell death. FEBS Letters 459, 421-426.

Alzheimer A. (1907) Uber eine eigematige Erkrankung der Himrinde. Allg. Z Psychiatr. Psychisch-Gericht. Med. 64, 146-148 .

Anderson AJ, Cummings BJ and Cotman CW. (1994) Increased immunoreactivity for Jun- and Fos-related proteins in Alzheimers disease. Exp Neurol 125, 286-295.

Anderson AJ, Su JH and Cotman CW. (1996) DNA damage and apoptosis in Alzheimers disease: colocalization with c-Jun immunoreactivity, relationship to brain area and effect of postmortem delay. J. Neurosci 16, 1710-1719.

Ando Y, Brannstrom T, Uchida K, Nyhlin N, Nasman B, Suhr O, Yamashita T, Olsson T, El Salhy M, Uchino M and Ando M. (1998) Histochemical detection of 4-hydroxynonenal protein in Alzheimer amyloid. J Neurol Sci,156:172.

Araujo DM, Lapchak PA, Robitaille Y, Gauthier S and Quirion R. (1988). Differential alteration of various cholinergic markers in cortical and subcortical regions of human brain in Alzheimer's disease. J. Neurochem. 50, 1914–1923.

Artola A and Singer W. (1993) Long-term depression of excitatory synaptic transmission and its relationship to long-term potentiation. Trends in Neuroscience, 16, 480–487.

Ascher P and Nowak L. (1988) The role of divalent cations in the N-methyl-D-aspartate responses of mouse central neurones in culture. J. Physiol. (London) m: 247-266.

Atkins CM, Selcher JC, Petraitis JJ, Trzaskos JM and Sweatt JD. (1998) The MAPK cascade is required for mammalian associative learning. Nat. Neurosci. 1, 602–609.

Atwood CS, Moir RD, Huang X, Scarpa RC, Bacarra NM, Romano DM, Hartshorn MA, Tanzi RE and Bush AI. (1988). Dramatic aggregation of Alzheimer Abeta by Cu^{2+} is induced by conditions representing physiological acidosis. J Biol Chem;273:12817.

Aubert I, Cecyre D, Gauthier S and Quirion R. (1996) Comparative ontogenic profile of cholinergic markers, including nicotinic and muscarinic receptors, in the rat brain. J. Comp. Neurol. 369, 31–55.

Aubert I, Araujo DM, Cecyre D, Robitaille Y, Gauthier S and Quirion R. (1992) Comparative alterations of nicotinic and muscarinic binding sites in Alzheimer's and Parkinson's diseases. J. Neurochem. 58, 529–541.

Auld DS, Mennicken F, Day JC and Quirion R. (2001a) Neurotrophins differentially enhance acetylcholine release, acetylcholine content and choline acetyltransferase activity in basal forebrain neurons. J. Neurochem. 77, 253–262.

Auld DS, Mennicken F and Quirion R. (2001b) Nerve growth factor rapidly induces prolonged acetylcholine release from cultured basal forebrain neurons: differentiation between neuromodulatory and neurotrophic influences. J. Neurosci. 21, 3375–3382.

Bamberger ME, Harris ME, McDonald DR, Husemann J and Landreth GE. (2003) A cell surface receptor complex for fibrillar β-amyloid mediates microglial activation. J. Neuroscience 23, 2665-2674.

Barone FC and Parsons AA. (2000) Therapeutic potential of anti-inflammatory drugs in focal stroke. Expert Opin Investig Drugs. Oct;9(10):2281-306.

Barr RK, Kendrick TS and Bogoyevitch MA. (2002) Identification of the critical features of a small peptide inhibitor of JNK activity. J Biol Chem 277, 10987-97.

Barrow CJ, Yasuda A, Kenny PT and Zagorski MG. (1992) Solution conformations and aggregational properties of synthetic amyloid beta-peptides of Alzheimer's disease. Analysis of circular dichroism spectra. J Mol Biol. Jun 20;225(4):1075-93.

Barrow C and Zagorski M. (1991) Solution structures of beta- peptide and its constituent fragments : relation to amyloid deposition. Science 253, 179-182.

Bashir ZI, Bortolotto ZA, Davies CH, Berretta N, Irving AJ, Seal AJ, Henley JM, Jane DE, Watkins JC and Collingridge GL. (1993) Induction of LTP in the hippocampus needs synaptic activation of glutamate metabotropic receptors. Nature. May 27;363(6427):347-50.

Beckman JS. (1996) Oxidative damage and tyrosine nitration from peroxynitrite. Chem Res Toxicol;9:836.

Behnish T, Fedorov K and Reymann KG (1991) L-2-Amino-3-phosphonopropionate blocks late synaptic long-term potentiation. NeuroReport 2: 386-388.

Behnish T and Reymann KG (1993) Coactivation of metabotropic glutamate and N-methyl-Daspartate receptors is involved in mechanisms of long-term potentiation maintenance in rat hippocampal CA1 neurons. Neurosci. 2: 37-48.

Bennett BL, Sasaki DT, Murray BW, O'Leary EC, Sakata ST, Xu W, Leisten JC, Motiwala A, Pierce S, Satoh Y, Bhagwat SS, Manning AM and Anderson DW. (2001) SP600125, an anthrapyrazolone inhibitor of Jun N-terminal kinase. Proc Natl Acad Sci U S A., 98, 13681-6

Berman DE, Hazvi S, Rosenblum K, Seger R and Dudai Y. (1998) Specific and differential activation of mitogen-activated protein kinase

cascades by unfamiliar taste in the insular cortex of the behaving rat. J Neurosci. Dec 1;18(23):10037-44.

Bi X, Gall CM, Zhou J and Lynch G (2002) Uptake and pathogenic effects of amyloid β-peptide 1-42 are enhanced by integrin antagonists and blocked by NMDA receptor antagonists. Neuroscience 112, 827-840.

Bitan G, Kirkitadze MD, Lomakin A, Vollers SS, Benedek GB and Teplow DB (2003) Amyloid β-protein (Aβ) assembly: A β1-40 and A β1-42 oligomerize through distinct pathways. Proc Natl Acad Sci U S A 7, 330-5

Blanchard BJ, Thomas VL and Ingram VM. (2002) Mechanism of membrane depolarisation caused by the Alzheimer A β 1-42 peptide. Biochemical and Biophysical Research Communications 293, 1197-1203.

Bliss TV and Collingridge GL. (1993) A synaptic model for memory: Long-term potentiation in the hippocampus. Nature 361: 3 1-39.

Bliss TV and Gardner-Medwin AR. Long-lasting potentiation of synaptic transmission in the dentate area of the unanaestetized rabbit following stimulation of the perforant path. J Physiol. 1973 Jul;232(2):357-74.

Bliss TV and Lomo T. (1973) Long-lasting potentiation of synaptic transmission in the dentate area of the anaesthetized rabbit following stimulation of the perforant path. J Physiol. Jul;232(2):331-56.

Bliss TV and Lynch MA, (1988) Long-lasting potentiation of synaptic transmission in the hippocampus: properties and mechanisms. In

Neurology and Neurobiology. Vol 35 , long term potentiation: From bio physics to behavior . by Landfield PW and Deadwyler SA, Alan R. Liss. New York pp3-71.

Blum S, Moore AN, Adams F and Dash PK. (1999) A mitogen-activated protein kinase cascade in the CA1/CA2 subfield of the dorsal hippocampus is essential for long-term spatial memory. J Neurosci. May 1;19(9):3535-44.

Bolaños JP and Medina JM. (1996) Induction of NOS inhibits gap junction permeability in cultures of rat astrocytes. J Neurochem;66:2091.

Bolshakov VY, Carboni L, Cobb MH, Siegelbaum SA and Belardetti F. (2000) Dual MAP kinase pathways mediate opposing forms of long-term plasticity at CA3-CA1 synapses. Nat Neurosci. 3, 1107-12.

Bonny C, Oberson A, Negri S, Sauser C and Schorderet DF. (2001) Cell-permeable peptide inhibitors of JNK: novel blockers of □ β-cell death. Diabetes 50, 77-82

Bortolotto ZA and Collingridge GL. (1993) Characterisation of LTP induced by the activation of glutamate metabotropic receptors in area CA1 of the hippocampus. Neuropharmacol. 32:1-9.

Bortolotto ZA and Collingridge GL. (1995) On the mechanism of long-term potentiation induced by (1S,3R)-1-aminocyclopentane-1,3-dicarboxylic acid (ACPD) in rat hippocampal slices. Neuropharmacology. 1995 Aug;34(8):1003-14.

Boyzyzcko-Coyne D, Horstmenn S, Anneser JMH, Neff NT and Glicksman MA. (2001) CEP-1347/KT-7515, an inhibitor of SAPK/JNK pathway activation, promotes survival and blocks multiple events associated with A β -induced cortical neuron apoptosis. J. Neurosci 77, 849-863.

Bozeat S, Gregory CA, Ralph MA and Hodges JR. (2000) Which neuropsychiatric and behavioural features distinguish frontal and temporal variants of frontotemporal dementia from Alzheimer's disease? J Neurol Neurosurg PsychiatryAug;69(2):178-86.

Braham CR and Srebro B. (1987) Induction of long-term depression and potentiation by low and high frequency stimulation in the dentate areas of the anaesthetized rat. Brain Research, 405, 100–107.

Brandan E and Inestrosa N. (1993) Extracellular matrix components and amyloid in neuritic plaques of Alzheimer's disease . Gen. Pharmacol. 24, 1063-1068 .

Breakwell NA, Rowan MJ and Anwyl R. (1996) Metabotropic glutamate receptor dependent EPSP and EPSP-spike potentiation in area CA1 of the submerged rat hippocampal slice. J Neurophysiol. Nov;76(5):3126-35.

Breakwell NA, Rowan MJ and Anwyl R. (1998) (+)-MCPG blocks induction of LTP in CA1 of rat hippocampus via agonist action at an mGluR group II receptor. J Neurophysiol. Mar;79(3):1270-6.

Brecht S, Simler S, Vergnes M, Mielke K, Marescaux C and Herdegen T. (1999) Repetitive electroconvulsive seizures induce activity of c-Jun N-

terminal kinase and compartment-specific desensitization of c-Jun phosphorylation in the rat brain. Brain Res Mol Brain Res. May 7;68(1-2):101-8.

Brown TH, Kairiss EW and Kernan CC. (1990) Hebbian synapses: biophysical mechanisms and algorithms. Annual Review of Neuroscience, **13**, 475–511.

Bruno V, Ksiazek, I, Battaglia G, Lukic S, Leonhardt T, Sauer T, Sauer D, Gasparini F, Kuhn R, Nicoletti F and Flor PJ. (2000) Selective blockade of metabotropic glutamate receptor subtype 5 is neuroprotective. Neuropharmacology 39, 2223-2230.

Bruno V, Battaglia G, Copani A, Sortino MA, Canonico PL and Nicoletti F. (1994) Protective action of idebenone against excitotoxic degeneration in cultured cortical neurons. Neuroscience Lett;178:193.

Burdick D, Soreghan B, Kwon M, Kosmoski J, Knauer M, Henschen A, Yates J, Cotman C and Glabe C. (1992) Assembly and aggregation properties of synthetic Alzheimer's A4/beta amyloid peptide analogs. J Biol Chem. Jan 5;267(1):546-54.

Bush AI, Pettingell WH, Multhaup G, d Paradis M, Vonsattel JP, Gusella JF, Beyreuther K, Masters CL and Tanzi RE. (1994) Rapid induction of Alzheimer A beta amyloid formation by zinc. Science. Sep 2;265(5177):1464-7.

Catania A, Urban S, Yan E, Hao C and Barron G, (2001) Allalunis-

Turner J. Expression and localization of cyclin-dependent kinase 5 in apoptotic human glioma cells. Neuro-oncol. Apr;3(2):89-98.

Cavus I and Teyler T (1996) Two forms of long-term potentiation in area CA1 activate different signal transduction cascades. J. Neurophysiology 76, 3038.

Chang L and Karin M (2001) Mammalian MAP kinase signalling cascades. Nature 410, 37-40.

Chao CC, Hu S, Sheng WS, Bu D, Bukrinsky MI and Peterson PK. (1996) Cytokine-stimulated astrocytes damage human neurons via a nitric oxide mechanism. Glia;16:276.

Chapman PF, White GL, Jones MW, Cooper-Blacketer D, Marshall VJ, Irizarry M, Younkin L, Good, MA, Bliss TVP, Hyman BT, Younkin SG and Hsiao KK (1999). Impaired synaptic plasticity and learning in aged amyloid precursor protein transgenic mice. Nature Neuroscience 2, 271-276.

Chen Q, Kagan BL, Hirajura Y and Xie C (2000) Impairments of hippocampaml long-term potentiation by Alzheimer β-peptide. J. Neurosci Res 60, 65-72.

Chou P and Fasman G. (1978) Empirical predictions of protein conformation . Annu. Rev. Biochem . 47, 251-276.

Clarke PB. (1995) Nicotinic receptors and cholinergic neurotransmission in the central nervous system. Ann. N. Y. Acad. Sci. 757, 73–83.

Coffey ET, Hongisto V, Dickens M, Davis RJ and Courtney MJ. (2000) Dual roles for c-Jun N-terminal kinase in developmental and stress responses in cerebellar granule neurons. J Neurosci 20, 7602-13

Collingridge GL, Kehl SJ and McLennan H. (1983) Excitatory amino acids in synaptic transmission in the Schaffer collateral-commissural pathway of the rat hippocampus. J. Physiol. (London) 342: 33-46.

Combs CK, Johnson DJ, Cannady SB, Lehman TM and Landreth GE (2000). Inflammatory mechanism in Alzheimers disease: inhibition of □ β-amyloid-stimulated proinflammatory responses and neurotoxicity by PPAR-gamma agonist. J. Neurosci 21, 1179-1181.

Coogan AN, O'Neill LA and O'Connor JJ. (1999) The P38 mitogen-activated protein kinase inhibitor SB203580 antagonizes the inhibitory effects of interleukin-1beta on long-term potentiation in the rat dentate gyrus in vitro. Neuroscience. 93(1):57-69.

Costello DA, Herron CE. The role of c-Jun N-terminal kinase in the A beta-mediated impairment of LTP and regulation of synaptic transmission in the hippocampus. Neuropharmacology. 2004 Apr;46(5):655-62.

Cotman CW and Su JH. (1996) Mechanisms of neuronal death in Alzheimer's disease. Brain Pathol. 4, 493-506.

Court JA, Martin-Ruiz C, Graham A and Perry E. (2000) Nicotinic receptors in human brain: topography and pathology. J. Chem. Neuroanat. 20, 281–298.

Coyle JT and Puttfarcken P. (1993) Oxidative stress, glutamate and neurodegenerative disorders. Science;262:689.

Crews CM, Alessandrini A and Erikson RL. (1992) The primary structure of MEK, a protein kinase that phosphorylates the ERK gene product. Science 258:478–480.

Cullen WK, Suh Y-H, Anwyl R and Rowan MJ (1997). Block of LTP in rat hippocampus in vivo by β -amyloid precursor protein fragments. NeuroReport 8, 3213-3217.

Cullen WK, Wu J, Anwyl R and Rowan MJ. (1996) β-Amyloid produces a delayed NMDA receptor-dependent reduction in synaptic transmission in rat hippocampus. NeuroReport 8, 87–92.

Dahlgren KN, Manelli AM, Stine WB Jr, Baker LK, Krafft GA and LaDu MJ. (2002) Oligomeric and fibrillar species of amyloid- β peptides differentially affect neuronal viability. J. Biol Chem 277, 32046-53

Davis S., Vanhoutte P., Pages C., Caboche J. and Laroche S. (2000) The MAPK/ERK cascade targets both Elk-1 and cAMP response element-binding protein to control long-term potentiationdependent gene expression in the dentate gyrus in vivo. J. Neurosci. 20, 4563±4572.

Dawson VL, Brahmbhatt HP, Mong JA and Dawson TM. (1994) Expression of inducible nitric oxide synthase causes delayed neurotoxicity in primary mixed neuronal-glial cortical cultures. Neuropharmacology 33:1425.

Derijard B, Raingeaud J, Barrett T, Wu IH, Han J, Ulevitch RJ and Davis RJ. (1995) Independent human MAP-kinase signal transduction pathways defined by MEK and MKK isoforms. Science. Feb 3;267(5198):682-5.

Derkinderen P, Enslen H and Girault JA. (1999) The ERK/MAP-kinases cascade in the nervous system. Neuroreport. Apr 6;10(5):R24-34.

DeSilva DR, Jones EA, Favata MF, Jaffee BD, Magolda RL, Trzaskos JM and Scherle P. A. (1998) Inhibition of mitogenactivated protein kinase kinase blocks T cell proliferation but does not induce or prevent anergy. J. Immunol. 160, 4175-4181.

Dhavan R and Tsai LH. (2001) A decade of CDK5. Nat Rev Mol Cell Biol, 10, 749-59

DiCamillo AM, Neff NT, Carswell S and Haun FA. (1998) Chronic sparing of delayed alternation performance and choline acetyltransferase activity by CEP-1347/KT-7515 in rats with lesions of nucleus basalis magnocellularis. Neuroscience. Sep;86(2):473-83.

Dickson DW, Crystal HA, Bevona C, Honer W, Vincent I and Davies P. (1995) Correlations of synaptic and pathological markers with cognition of the elderly. Neurobiol Aging 16, 285-98.

Dinerly KT, Westerman M, Bui D, Bell K, Ashe K and Sweatt JD (2001) β -amyloid activates the mitogen-activated protein kinase cascade via hippocampal □7 nicotinic acetylcholine receptors: in vitro and in vivo

mechanisms related to Alzheimers disease. J. Neuroscience 21, 4125-4133.

Dinerly KT, Bell K, Bui D and Sweatt JD (2001) β-Amyloid peptide activates alpha 7 nicotinic acetylcholine receptors expressed in Xenopus oocytes. J. Biol. Chem 277, 25056-25061.

Dobelis P, Staley KJ, Collins AC, Paylor R, Beaudet AL and Dunwiddie TV. (2002) Alpha-7 nicotinic mediated responses in the mouse dentate gyrus. Soc. Neurosci Abstr 37.9.

Dodart JC, Bales KR, Gannon KS, Greene SJ, DeMattos RB, Mathis C, DeLong CA, Wu S, Wu X, Holtzman DM and Paul SM. (2002) Immunization reverses memory deficits without reducing brain Abeta burden in Alzheimer's disease model. Nat Neurosci. 5(5):452-7.

Du Y, Ma Z, Lin S, Dodel RC, Gao F, Bales KR, Triarhou LC, Chernet E, Perry KW, Nelson DL, Luecke S, Phebus LA, Bymaster FP and Paul SM. (2001) Minocycline prevents nigrostriatal dopaminergic neurodegeneration in the MPTP model of Parkinson's disease. Proc. Natl. Acad. Sci. 98, 14669-14674.

Eikelenboom P, Zahn SS, van Gool WA and Allsop D. (1994) Inflammatory mechanisms in Alzheimer's disease. Trends Pharmacol Sci 15, 447-450.

Ekinci FJ, Malik KU and Shea TB. (1999) Activation of the L voltage-sensitive calcium channel by mitogen-activated protein (MAP) kinase following exposure of neuronal cells to beta-amyloid. MAP kinase

mediates beta-amyloid-induced neurodegeneration. J Biol Chem. Oct 15;274(42):30322-7.

English JD and Sweatt JD. (1996) Activation of p42 mitogen-activated protein kinase in hippocampal long term potentiation. J Biol Chem. Oct 4;271(40):24329-32.

English JD and Sweatt JD. (1997) Requirement for the mitogen-activated protein kinase cascade in hippocampal long term potentiation. J Biol Chem. Aug 1;272(31):19103-6.

Esch FS, Keim PS, Beattie EC, Blacher RW, Culwell AR, Oltersdorf T, McClure D, Ward PJ. (1990) Cleavage of amyloid beta peptide during constitutive processing of its precursor. Science. 1;248(4959):1122-4.

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

Ferrari-DiLeo G and Flynn DD. (1993) Diminished muscarinic receptor-stimulated [3H]-PIP2 hydrolysis in Alzheimer's disease. Life Sci. 53, PL439–PL444.

Ferrari-DiLeo G, Mash DC and Flynn DD. (1995) Attenuation of muscarinic receptor–G protein interaction in Alzheimer's disease. Mol. Chem. Neuropathol. 24, 69–91.

Fitzjohn SM, Bortolotto ZA, Palmer MJ, Doherty AJ, Ornstein PL, Schoepp DD, Kingston AE, Lodge D and Collingridge GL (1998) The potent mGluR receptor antagonist LY341495 identifies roles for both cloned and novel mGluR receptors in hippocampal synaptic plasticity. Neuropharmacology 37, 1455-1458.

Fitzjohn SM, Morton RA, Kuenzi F, Rosahl TW, Shearman M, Lewis H, Smith D, Reynolds DS, Davies CH, Collingridge GL and Seabrook GR. (2001) Age-related impairment of synaptic transmission but normal long-term potentiation in transgenic mice that overexpress the human APP695SWE mutant form of amyloid precursor protein. J. Neurosci. 21, 4691–4698.

Flood JF, Morley JE and Roberts E (1991) Amnestic Effects in Mice of Four Synthetic Peptides Homologous to Amyloid β-protein from patients with Alzheimer Disease. Proc Natl Acad Sci. 88, 3363-3366.

Francis PT, Palmer AM, Snape M and Wilcock GK. (1999) The cholinergic hypothesis of Alzheimer's disease: a review of progress. J Neurol Neurosurg Psychiatry. 66(2):137-47.

Fraser PE, Nguyen JT, Chin DT and Kirschner DA. (1992) Effects of sulfate ions on Alzheimer ß/A4 peptide assemblies : implications for amyloid fibril-proteoglycan interactions . J. Neurochem. 59, 1531-1540 .

Freir DB, Holscher C and Herron CE. (2001) Blockade of long-term potentiation by beta-amyloid peptides in the CA1 region of the rat hippocampus in vivo. J. Neurophysiol. 85, 708–713.

Frodin M and Gammeltoft S. (1999) Role and regulation of 90 kDa ribosomal S6 kinase (RSK) in signal transduction. Mol Cell Endocrinol. May 25;151(1-2):65-77.

Fukunaga R and Hunter T. (1997) MNK1, a new MAP kinase-activated protein kinase, isolated by a novel expression screening method for identifying protein kinase substrates.EMBO J. Apr 15;16(8):1921-33.

Garvey EP, Oplinger JA, Furfine ES, Kiff RJ, Laszlo F, Whittle BJ and Knowles RG. (1997) 1400W is a slow, tight binding, and highly selective inhibitor of inducible nitric-oxide synthase in vitro and in vivo. J Biol Chem 272, 4959-63.

Gasparini F, Lingenhöhl K, Stoehr N, Flor PJ, Heinrich M, Vranesic I, Biollaz M, Allgeier H, Heckendorn R, Urwyler S, Varney MA, Johnson EC, Hess SC, Sacaan AI, Santori EM, Veliçelebi G and Kuhn R (1999). 2-methyl-6-(phenylethynyl)pyridine (MPEP), a potent selective and systemically active mGluR5 antagonist. Neuropharmacology 38, 1493-1503.

Glenner G. (1980) Amyloid deposits and amyloidosis : the fibrilloses. N. Engl. J. Med. 302, 1283-1292 .

Glenner GG and Wong CW. (1984) Alzheimer's disease and Down's syndrome: sharing of a unique cerebrovascular amyloid fibril protein. Biochem Biophys Res Commun. 16;122(3):1131-5.

Glenner GG and Wong CW. (1984) Alzheimer's disease: initial report of the purification and characterization of a novel cerebrovascular amyloid protein. Biochem Biophys Res Commun. 16;120(3):885-90.

Goedert M. (1993) Tau protein and the neurofibrillary pathology of Alzheimer's disease, Trends Neurosci. Nov;16(11):460-5.

Good PF, Werner P, Hsu A, Olanow CW and Perl DP. (1996) Evidence of neuronal oxidative damage in Alzheimer's disease. Am J Pathol 149:21.

Gorevic PD, Castano EM, Sarma R and Frangione B. (1987) Ten to fourteen residue peptides of Alzheimer's disease protein are sufficient for amyloid fibril formation and its characteristic X-ray diffraction pattern . Biochem. Biophys. Res . Commun. 147, 854-862.

Gorevic PD, Goni F, Pons-Estel B, Alvarez F, Peress NS and Frangione B. (1986) Isolation and partial characterization of neurofibrillary tangles and amyloid plaque core in Alzheimer's disease: immunohistological studies. J Neuropathol Exp Neurol. 45(6):647-64.

Green KM and Peers C. (2001) Amyloid β - peptides mediate hypoxic augmentation of Ca^{2+} channels. J Neurochem 77:953.

Greenfield JP, Tsai J, Gouras GK, Hai B, Thinakaran G, Checler F, Sisodia SS, Greengard P and Xu H. (1999) Endoplasmic reticulum and trans-Golgi network generate distinct populations of Alzheimer beta-amyloid peptides. Proc Natl Acad Sci U S A. 19;96(2):742-7

Greenwood AF, Powers RE and Jope RS. (1995) Phosphoinositide hydrolysis, G alpha q, phospholipase C, and protein kinase C in post-mortem human brain: effects of post-mortem interval, subject age, and Alzheimer's disease. Neuroscience 69, 125–138.

Griffin WS, Sheng JC, Royston MC, Gentleman SM, McKenzie JE, Graham DI, Roberts GW and Mrak RE (1998) Glial-neuronal interactions in Alzheimer's disease: the potential role of a cytokine cycle in disease progression. Brain Pathol 8, 65-72.

Gray R, Rajan AS, Radcliffe KA, Yakehiro M and Dani JA. (1996) Hippocampal synaptic transmission enhanced by low concentrations of nicotine. Nature 383, 713–716.

Grover L. (1998) Evidence for postsynaptic induction and expression of NMDA receptor independent LTP. J. Neurophysiology 79, 1167-1182.

Grover L and Teyler TJ. (1990) Two components of long-term potentiaion induced by different patterns of afferent activation. Nature 347, 477-479.

Guan ZZ, Miao H, Tian JY, Unger C, Nordberg A and Zhang, X. (2001) Suppressed expression of nicotinic acetylcholine receptors by nanomolar beta-amyloid peptides in PC12 cells. J. Neural Transm. 108, 1417–1433.

Guan ZZ, Zhang X, Ravid R and Nordberg,A. (2000) Decreased protein levels of nicotinic receptor subunits in the hippocampus and temporal cortex of patients with Alzheimer's disease. J. Neurochem. 74, 237–243.

Haass C, Hung AY, Selkoe DJ. (1991) Processing of beta-amyloid precursor protein in microglia and astrocytes favors an internal localization over constitutive secretion. J Neurosci. 11(12):3783-93.

Halverson K, Fraser P, Kirschner D and Lansbury TJ. (1990) Molecular determinants of amyloid deposition in Alzheimer's disease : conformational studies of synthetic 0-protein fragments. Biochemistry 29, 2639-2644 .

Han G and Hampson DR. (1999) Ligand binding to the amino-terminal domain of the mGluR4 subtype of metabotropic glutamate receptor. J Biol Chem. Apr 9;274(15):10008-13.

Hanks SK and Hunter T. (1995) Protein kinases 6. The eukaryotic protein kinase superfamily: kinase (catalytic) domain structure and classification. FASEB J 9:576–596.

Hardy JA and Higgins GA. (1992) Alzheimer's disease: the amyloid cascade hypothesis. Science. 10;256(5054):184-5.

Hardy J and Selkoe DJ. (2002) The amyloid hypothesis of Alzheimers disease:progress and problems on the road to therapeutics. Science 297, 353-356.

Harper JD and Lansbury PT. (1997) Models of amyloid seeding in Alzheimer's disease and scrapie: mechanistic truths and physiological consequences of the time-dependent solubility of amyloid proteins. Annu Rev Biochem. 66:385-407.

Harper SJ and LoGrasso P. (2001) Signalling for survival and death in neurones: the role of stress-activated kinases, JNK and p38. Cell Signal. May;13(5):299-310.

Harper JD, Wong SS, Lieber CM and Lansbury PT. (1997) Observation of metastable Abeta amyloid protofibrils by atomic force microscopy. Chem Biol. 1997 Feb;4(2):119-25. Chem. Biol. 4, 119-125.

Harris ME, Wang Y, Pedigo NW, Hensley K and Butterfield DA, Carney JM. (1998) Amyloid beta peptide (25–35) inhibits Na+- dependent glutamate uptake in rat hippocampal astrocyte cultures. J Neurochem 67:277.

Harris EW and Cotman CW. (1986) Long-term potentiation of guinea pig mossy fibre responses is not blocked by N-methyl-Daspartate antagonists. Neurosci. Lett. 211: 132-137.

Hartley DM, Walsh DM, Ye CP, Diehl T, Vasquez S, Vassilev PM, Teplow DB and Selkoe DJ. (1999) Protofibrillar intermediates of amyloid β -protein induce acute electrophysiological changes and progressive neurotoxicity in cortical neurons. J Neurosci 19, 8876-84.

Hensley K, Floyd RA, Zheng NY, Nael R, Robinson KA, Nguyen X, Pye ON, Stewart CA, Geddes J, Markesberry WR, Patel E, Johnson GVW and Bing G (1999). p38 kinase is activated in Alzheimer's disease brain. J. Neurochem 72, 2053-2058.

Hensley K, Maidt ML, Yu Z, Sang H, Markesbery WR and Floyd RA. (1998) Electrochemical analysis of protein nitrotyrosine and dityrosine in

the Alzheimer brain indicates region-specific accumulation. J Neurosci 18:8126.

Hering H and Sheng M. (2001) Dendritic spines: structure, dynamics and regulation. Nat Rev Neurosci 2: 880-888.

Hewett SJ, Csernansky CA and Choi DW. (1994) Selective potentiation of NMDA induced neuronal injury following induction of astrocytic iNOS. Neuron,13:487.

Hilbich C, Kisters-Woike B, Reed J, Masters C and Beyreuther K. (1991) Aggregation and secondary structure of synthetic amyloid ßA4 peptides of Alzheimer's disease . J. Mot. Biol. 218, 149-163.

Hirsch JC and Crepel F. (1992) Postsynaptic calcium is necessary for the induction of LTP and LTD of monosynaptic EPSPs in prefrontal neurons: an in vitro study on the induction of long-term potentiation. Science, 255, 730–733.

Holscher C, Anwyl R and Rowan MJ. (1997) Activation of group-II metabotropic glutamate receptors blocks induction of long-term potentiation and depotentiation in area CA1 of the rat in vivo. Eur J Pharmacol. Mar 19;322(2-3):155-63

Hodges JR and Patterson K. (1995) Is semantic memory consistently impaired early in the course of Alzheimer's disease? Neuroanatomical and diagnostic implications. Neuropsychologia. 33(4):441-59.

Hoshi M, Nishida E and Sakai H. (1988) Activation of a Ca2+ inhibitable protein kinase that phosphorylates microtubuleassociated protein 2 in vitro by growth factors, phorbol esters, and serum in quiescent cultured human Æbroblasts. J. Biol. Chem. 263, 5396-5401.

Hoshi M, Takashima A, Murayama M, Yasutake K, Yoshida N, Ishiguro K, Hoshino T and Imahori , K. (1997) Non-toxic amyloid beta-peptide 1–42 suppresses acetylcholine synthesis. Possible role in cholinergic dysfunction in Alzheimer's disease. *J. Biol. Chem.* 272 , 2038–2041.

Hoyer S. (1996) Oxidative metabolism deficiencies in brains of patients with Alzheimer's disease. Acta Neurolog Scand Suppl,165:18.

Hsia AY, Masliah E, McCologue L, Nicoll RA and Mucke L. (1999) Plaque-independent disruption of neural circuits in Alzheimer's disease mouse models. Proc. Natl Acad. Sci. USA 96, 3228–3233.

Hu J, Akama KT, Krafft GA, Chromy BA and Van Eldik LJ. (1998) Amyloid beta peptide activates cultured astrocytes: morphological alterations, cytokine induction and nitric oxide release. Brain Res;785:195.

Huang HM, Ou HC and Hsieh SJ. (2000) Amyloid beta-peptide impaired carbachol but not glutamate-mediated phosphoinositide pathways in cultured rat cortical neurons. Neurochem. Res. 25, 303–312.

Hyman BT, Marzloff K and Arriagada PV. (1993) The lack of accumulation of senile plaques or amyloid burden n Alzheimer's disease

suggests a dynamic balance between amyloid deposition and resolution. J Neuropathol Exp Neurol 52:594–600.

Iijima K, Ando K, Takeda S, Satoh Y, Seki T, Itohara S, Greengard P, Kirino Y, Nairn A and Suzuli T (2000) Neuron specific phosphorylation of Alzheimers □ β -amyloid precursor protein by cyclin-dependent kinase 5. J. Neurochemistry 75, 1085-1095.

Imahori K and Uchida T. (1997) Physiology and pathology of tau protein kinases in relation to Alzheimer's disease. J Biochem (Tokyo). Feb;121(2):179-88.

Impey S, Obrietan K, Wong ST, Poser S, Yano S, Wayman G, Deloulme JC, Chan G and Storm DR. (1998) Cross talk between ERK and PKA is required for Ca2+ stimulation of CREB-dependent transcription and ERK nuclear translocation. Neuron 21, 869-883.

Inestrosa NC and Soto C. (1992) Molecular biology of the amyloid of the Alzheimer's disease . Biol. Res. 25, 63-72.

Itoh A, Akaike T, Sokabe M, Nitta A and Nabeshima T (1999) Impairment of long-term potentiation in hippocampal slices of β - amyloid-infused rats. Eur. J. Pharmacol 3, 167-175.

Itoh, A., Nitta, A., Nadai, M., Nishimura, K., Hirose, M., Hasegawa, T., Nabeshima, T., 1996. Dysfunction of cholinergic and dopaminergic neuronal systems in beta-amyloid protein-infused rats. J. Neurochem. 66, 1113–1117.

Ito M. (1989) Long-term depression. Annual Review of Neuroscience, 12, 85–102.

Izumi Y, Clifford DB and Zorumski CF. (1991) 2-Amino-3-phosphonopropionate blocks the mductmn and maintenance of long-tam potentiation in hippocampal slices. Neurosci. Lett. 122: 187-191.

Jarrett JT, Berger EP and Lansbury PT. (1993) The C-terminus of the beta protein is critical in amyloidogenesis. Ann N Y Acad Sci. 24;695:144-8.

Jhamandas JH, Cho C, Jassar B, Harris KH, MacTavish D and Easaw J. (2001) Cellular mechanisms for amyloid beta-protein activation of rat cholinergic basal forebrain neurons. J. Neurophysiol. 86, 1312–1320.

Ji D, Lape R and Dani JA. (2001) Timing and location of nicotinic activity enhances or depresses hippocampal synaptic plasticity. Neuron 31, 131–141.

Jia Z, Lu Y, Henderson J, Taverna F, Romano C, Abramow-Newerly W, Wojtowicz JM and Roder J. (1998) Selective abolition of the NMDA component of long-term potentiation in mice lacking mGluR5. Learn Mem. Sep-Oct;5(4-5):331-43.

Jiang Y, Chen C, Li Z, Guo W, Gegner JA, Lin S and Han J. (1996) Characterization of the structure and function of a new mitogen-activated protein kinase (p38beta). J Biol Chem. Jul 26;271(30):17920-6.

Jiang Y, Gram H, Zhao M, New L, Gu J, Feng L, Di Padova F, Ulevitch

RJ and Han J. (1997) Characterization of the structure and function of the fourth member of p38 group mitogen-activated protein kinases, p38delta. J Biol Chem. Nov 28;272(48):30122-8.

Jiang H, Nurdick D, Glabe GG, Cotman CW and Tenner AJ. (1994) β–Amyloid activates complement by binding to a specific region of the collagenlike domain of the C1q A chain. J Immunol;152:5050.

Jones S, Sudweeks S and Yakel JL. (1999) Nicotinic receptors in the brain: correlating physiology with function. Trends Neurosci. 22, 555–561.

Jope RS, Song L, Li X and Powers R. (1994) Impaired phosphoinositide hydrolysis in Alzheimer's disease brain. Neurobiol. Aging 15, 221– 226.

Jope RS, Song L and Powers RE. (1997) Cholinergic activation of phosphoinositide signaling is impaired in Alzheimer's disease brain. Neurobiol. Aging 18, 111–120.

Kamsler A and Segal M. (2003) Paradoxical actions of hydrogen peroxide on long-term potentiation in transgenic superoxide dismutase-1 mice. J Neurosci 23, 10359-67.

Kang J, Lemaire HG, Unterbeck A, Salbaum JM, Masters CL, Grzeschik KH, Multhaup G, Beyreuther K and Muller-Hill B. (1987) The precursor of Alzheimer's disease amyloid A4 protein resembles a cell-surface receptor. Nature. 19-25;325(6106):733-6.

Kano M and Kato M. (1987) Quisqualate receptors are specifically involved in cerebellar synaptic plasticity. Nature, 325, 276–279.

Kar S, Seto D, Gaudreau P and Quirion , R. (1996) Beta-amyloid-related peptides inhibit potassium-evoked acetylcholine release from rat hippocampal slices. J. Neurosci. 16 ,1034–1040.

Kar S, Issa AM, Seto D, Auld DS. Collier B and Quirion, R. (1998) Amyloid beta-peptide inhibits high-affinity choline uptake and acetylcholine release in rat hippocampal slices. J. Neurochem. 70 ,2179–2187.

Katzman R. (1986) Alzheimer's disease. N Engl J Med. 10;314 (15): 964-73.

Kayed R, Head E, Thompson JL, McIntire TM, Milton SC, Cotman CW and Glabe CG. (2003) Common structure of soluble amyloid oligomers implies common mechanism of pathogenesis. Science. 300, 486-9

Keller JN, Pang Z, Geddes JW, Begley JG, Germeyer A, Waeg G and Mattson MP. (1997) Impairment of glucose and glutamate transport and induction of mitochondrial oxidative stress and dysfunction in synaptosomes by amyloid beta-peptide: role of the lipid peroxidation product 4-hydroxynonenal. J Neurochem;69:273.

Kelly JF, Furukawa K, Barger SW, Rengen MR, Mark RJ, Blanc EM, Roth GS and Mattson MP. (1996) Amyloid beta-peptide disrupts carbachol-induced muscarinic cholinergic signal transduction in cortical neurons. Proc. Natl. Acad. Sci. U.S.A. 93, 6753–6758.

Kim JH, Anwyl R, Suh YH, Djamgoz MB and Rowan MJ. (2001) Use-dependent effects of amyloidogenic fragments of β-amyloid precursor protein on synaptic plasticity in rat hippocampus in vivo. J. Neurosci. 21, 1327–1333.

Kingston AE, Ornstein PL, Wright RA, Johnson BG, Mayne NG, Burnett JP, Belagaje R, Wu S and Schoepp DD (1998) LY341495 is a nanomolar potent and selective antagonist of group II metabotropic glutamate receptors. Neuropharmacology 37, 1-12.

Kirschner D, Abraham C, and Selkoe D. (1986) X-ray diffraction from intraneuronal paired helical filaments and extraneuronal amyloid fibers in Alzheimer disease indicates cross-/J conformation. Proc . Natl. Acad. Sci. USA 83, 503-507.

Klann E, Robertson ED, Knapp LT and Sweatt JD (1998) A role for superoxide in protein kinase C activation and induction of long-term potentiation. J. Biol. Chem 273, 4516-4522.

Klein WL, Krafft GA and Finch CE. (2001) Targeting small Abeta oligomers: the solution to an Alzheimer's disease conundrum? Trends Neurosci. 24(4):219-24.

Kluck RM, Bossy-Wetzel E, Green DR and Newmeyer DD. (1997) The release of cytochrome c from mitochondria: a primary site for Bcl-2 regulation of apoptosis. Science;275:1132.

Klyubin I, Walsh DM, Cullen WK, Fadeeva JV, Anwyl R, Selkoe DJ and

Rowan MJ. (2004) Soluble Arctic amyloid beta protein inhibits hippocampal long-term potentiation in vivo. Eur J Neurosci. May;19(10):2839-46.

Knipper M, da Penha Berzaghi M, Blochl A, Breer H, Thoenen H and Lindholm D. (1994) Positive feedback between acetylcholine and the neurotrophins nerve growth factor and brain-derived neurotrophic factor in the rat hippocampus. Eur. J. Neurosci. 6, 668–671.

Knockaert M, Greengard P and Meijer L. (2002) Pharmacological inhibitors of cyclin-dependent kinases. Trends in Pharmacological Sciences 23, 417-425.

Koo EH and Squazzo SL. (1994) Evidence that production and release of amyloid beta-protein involves the endocytic pathway. J Biol Chem. 1;269(26):17386-9.

Kosik K. (1991) Alzheimer plaques and tangles : advances on both fronts . Trends Neurosci. 14, 218-219.

Kosik KS, Joachim CL and Selkoe DJ. (1986) Microtubule-associated protein tau (tau) is a major antigenic component of paired helical filaments in Alzheimer disease. Proc Natl Acad Sci U S A. Jun;83(11):4044-8.

Krištofiková Z, Tejkalová H and Klaschka J. (2001) Amyloid beta-peptide 1–40 and the function of rat hippocampal hemicholinium-3 sensitive choline carriers: effects of a proteolytic degradation in vitro. Neurochem. Res. 26, 203–213.

Kuo YM, Emmerling MR, Vigo-Pelfrey C, Kasunic TC, Kirkpatrick JB, Murdoch GH, Ball MJ and Roher AE. (1996) Water-soluble Abeta (N-40, N-42) oligomers in normal and Alzheimer disease brains. J. Biol. Chem. 271, 4077–4081

Kuperstein F and Yavin E. (2002) ERK activation and nuclear translocation in amyloid-beta peptide- and iron-stressed neuronal cell cultures. Eur J Neurosci. Jul;16(1):44-54.

Kyriakis JM and Avruch J. (2001) Mammalian mitogen-activated protein kinase signal transduction pathways activated by stress and inflammation. Physiol Rev. Apr;81(2):807-69.

Kyriakis JM, Banerjee P, Nikolakaki E, Dai T, Rubie EA, Ahmad MF, Avruch J and Woodgett JR. (1994) The stressactivated protein kinase subfamily of c-Jun kinases. Nature 369, 156 – 160.

Lambert MP, Barlow AK, Chromy BA, Edwards C, Freed R, Liosatos M, Morgan TE, Rozovsky I, Trommer B, Viola KL, Wals P, Zhang C, Finch CE, Krafft GA and Klein WL. (1998) Diffusible nonfibrillar ligands derived from A β 1-42 are potent central nervous system neurotoxins. Proc Natl Acad Sci 95, 6448-6453.

Lansbury PT (1992) In pursuit of the molecular structure of amyloid plaque : new technology provides unexpected and critical information. J. Biochemistry 31, 6865-6870 .

Laszlo F, Evans SM and Whittle BJ. (1995) Aminoguanidine inhibits both constitutive and inducible nitric oxide synthase isoforms in rat intestinal microvasculature in vivo. Eur J Pharmacol 272, 169-75.

Lawrence AD and Sahakian BJ. (1998) The cognitive psychopharmacology of Alzheimer's disease: focus on cholinergic systems. Neurochem Res. 23(5):787-94.

Lee JC, Laydon JT, McDonnell PC, Gallagher TF, Green D, McNulty D, Blumenthal MJ, Heys RJ, Landvatter SW, Stricker JE, McLaughlin MM, Siemens I, Fisher S, Livi GP, White JR, Adams JL and Young PR (1994) Identification and characterization of a novel protein kinase involved in the regulation of inflammatory cytokine biosynthesis. Nature 372, 739-746.

Lee MS, Kwon YT, Li M, Peng J, Friedlander RM and Tsai LH (2000) Neurotoxicity induces cleavage of p35 to p25 by calpain. Nature 405, 360-364.

Lee T, Shiao YJ, Chen CF and Wang LC. (2001) Effect of ginseng saponins on beta-amyloid-suppressed acetylcholine release from rat hippocampal slices. Planta Med. 67, 634–637.

Levin ED and Simon BB. (1998) Nicotinic acetylcholine involvement in cognitive function in animals. Psychopharmacology (Berl.) 138, 217–230.

Levy E, Carman M, Fernandez-Madrid I, Power M, Lieberburg I, van Duinen S, Gerard T, Bots A, Luyendijk W and Frangione B. (1990)

Mutation of the Alzheimer's disease amyloid gene in hereditary cerebral hemorrhage, Dutch type . Science 248, 1124-1128 .

Levy WB and Steward O. (1979) Synapses as associative memory elements in the hippocampal formation. Brain Research, 175, 233–245.

Liberatore GT, Jackson-Lewis V, Vukosavic S, Mandir AS, Vila M, McAuliffe WG, Dawson VL, Dawson TM and Przedborski S.(1999) Inducible nitric oxide synthase stimulates dopaminergic neurodegeneration in the MPTP model of Parkinson disease. Nat Med;5:1403.

Li R, Yang L, Lindholm K, Konishi Y, Yue X, Hampel H, Zhang D and Shen Y. (2004) Tumor necrosis factor death receptor signaling cascade is required for amyloid-beta protein-induced neuron death. J Neurosci. 24, 1760-71.

Li Z, Jiang Y, Ulevitch RJ and Han J. (1996) The primary structure of p38 gamma: a new member of p38 group of MAP kinases. Biochem Biophys Res Commun. Nov 12;228(2):334-40.

Linden DJ. (1994) Long-term synaptic depression in the mammalian brain. Neuron, 12, 457–472.

Lipton SA, Choi YB, Pan ZH, Lei SZ, Chen HS, Sucher NJ, Loscalzo J, Singel DJ and Stamler JS (1993) A redox-based mechanism for the neuroprotective and neurodestructive effects of nitric oxide and related nitroso-compounds. Nature. 364, 626-32

Lipton SA and Rosenberg PA. (1994) Excitatory amino acids as a final common pathway for neurologic disorders. N Engl J Med;330:613.

Lisman J. (1989) A mechanism for the Hebb and anti-Hebb processes underlying learning and memory. Pro-ceedings of the National Academy of Sciences of the USA, **86**,9574–9578.

Lisman J, Schulman H and Cline H (2002) The molecular basis of CaMKII function in synaptic and behavioural memory. Nat Rev Neurosci 3, 175-190.

Liu F, Ma X-H, Ule J, Bibb JA, Nishi A, DeMaggio AJ, Yan Z, Nairn AC and Greengard P (2001) Regulation of cyclin-dependent kinase 5 and casein kinase 1 by metabotropic glutamate receptors. Proc. Natl. Acad. Sci 98, 11062-11068.

Liu Q, Kawai H and Berg DK. (2001) Beta-amyloid peptide blocks the response of alpha7-containing nicotinic receptors on hippocampal neurons. Proc. Natl. Acad. Sci. U.S.A. 98, 4734–4739.

Lorenzo A and Yankner BA (1994) β-amyloid neurotoxicity requires fibril formation and is inhibited by congo red. Proc. Natl. Acad. Sci 91, 12243-12247.

Lovinger DM, Tyler EC and Merritt AJ. (1993) Short- and long-term synaptic depression in rat neostriatum. Neurophyisiology, 70, 1937–1949.

Lue LF, Kuo YM, Roher AE, Brachova L, Shen Y, Sue L, Beach T, Kurth JH, Rydel RE and Rogers J. (1999) Soluble amyloid peptide concentration as a predictor of synaptic change in Alzheimer's disease. Am J Pathol., 155, 853-62.

Maccioni RB and Cambiazo V. (1995) Role of microtubule-associated proteins in the control of microtubule assembly. Physiol Rev. Oct;75(4):835-64.

MacKenzie IR and Munoz DG. (1998) Nonsteroidal anti-inflammatory use and Alzheimer-type pathology in ageing. Neurology 50, 986-990.

MacManus A, Ramsden M, Murray M, Henderson Z, Pearson H and Campbell V. (2000) Enhancement of 45Ca2+ influx and voltage dependent Ca2+ channel activity by β-amyloid (1–40) in rat cortical synaptosomes and cultured cortical neurons. J Biol Chem;275:713.

Manahan-vaughan D and Reymann G. (1995) Regional and developmental profile of modulation in hippocampal synaptic transmission and LTP by AP4-sensitive mGluRs in VIVO. Neuropharmacol. 2: 991-100 .

Mandelkow EM, Biernat J, Drewes G, Gustke N, Trinczek B and Mandelkow E. (1995) Tau domains, phosphorylation, and interactions with microtubules. Neurobiol Aging. May-Jun;16(3):355-62; discussion 362-3.

Mansvelder HD and McGehee DS. 2000. Long-term potentiation of excitatory inputs to brain reward areas by nicotine. Neuron 27, 349–357.

Manzoni OJ, Weisskopf MG and Nicoll RA. (1994) MCPG antagonizes metabotropic glutamate receptors but not long-term potentiation in the hippocampus. Eur J Neurosci. 1994 Jun 1;6(6):1050-4.

Marin DB, Sewell MC and Schlechter A. (2002) Alzheimer's disease. Accurate and early diagnosis in the primary care setting. Geriatrics. 57(2):36-40; quiz 43.

Mark RJ, Blanc EM and Mattson MP. (1996) Amyloid beta-peptide and oxidative cellular injury in Alzheimer's disease. Mol Neurobiol; 12:211.

Martin SJ, Grimwood PD and Morris RG. (2000) Synaptic plasticity and memory: an evaluation of the hypothesis. Annu. Rev. Neurosci. 23, 649–711.

Martin SJ and Morris RG. (2002) New life in an old idea: the synaptic plasticity and memory hypothesis revisited. Hippocampus. 12, 609-36.

Martin-Ruiz CM, Court JA, Molnar E, Lee M, Gotti C, Mamalaki A, Tsouloufis T, Tzartos S, Ballard C, Perry RH and Perry EK. (1999) Alpha4 but not alpha3 and alpha7 nicotinic acetylcholine receptor subunits are lost from the temporal cortex in Alzheimer's disease. J. Neurochem. 73, 1635–1640.

Masters CL, Simms G, Weinman NA, Multhaup G, McDonald BL and Beyreuther K. (1985) Amyloid plaque core protein in Alzheimer disease and Down syndrome. Proc Natl Acad Sci U S A. 82(12):4245-9.

Mattson MP. (1997) Cellular actions of _ β -amyloid precursor protein and its soluble and fibrillogenic peptide derivates. Physiol Rev; 77:1081.

Maurice T, Lockart BP and Privat A (1996) Amnesia induced in mice by centrally administered β-amyloid peptides involves cholinergic dysfunction. Brain Res 706, 181-193.

Mayeux R and Sano M. (1999) Treatment of Alzheimer's disease. N Engl J Med;34:1670.

McDonald DR, Brunden KR and Landreth GE (1997) Amyloid fibrils activate tyrosine kinase-dependnet signalling and superoxide production in microglia. J. Neurosci 17, 2284-2288.

McDonald DR, Bamberger ME, Combs CK and Landreth GE (1998) β-amyloid fibrils activate parallel mitogen-activated protein kinase pathways in microglia and THP1 monocytes. J. Neurosci 18, 4451-4460.

McGahon B, Maguire C, Kelly A and Lynch MA. (1999) Activation of p42 mitogen-activated protein kinase by arachidonic acid and trans-1-amino-cyclopentyl-1,3-dicarboxylate impacts on long-term-potentiation in the dentate gyrus in the rat: analysis of agrelated changes. Neuroscience 90, 1167-1175.

McGeer PL and McGeer EG (1995) The inflammatory response system of the brain: implications for therapy of Alzheimer and other neurodegenerative diseases. Brain Res Rev 21, 195-218.

McGeer EG and McGeer PL. (1999) Brain inflammation in Alzheimer disease and the therapeutic implications. Curr Pharm Des. Oct;5(10):821-36.

McGeer EG and McGeer PL. (2003) Inflammatory processes in Alzheimer's disease. Prog Neuropsychopharmacol Biol Psychiatry. 27, 741-9

McGuinness N, Anwyl R and Rowan M. (1991) Trans-ACPD enhances long-term potentiation in the hippocampus. Eur J PharmacolMay 17;197(2-3):231-2.

McLean CA, Cherny RA, Fraser FW, Fuller SJ, Smith MJ, Beyreuther K, Bush AI and Masters CL. (1999) Soluble pool of Abeta amyloid as a determinant of severity of neurodegeneration in Alzheimer's disease. Ann. Neurol. 46, 860–866

Mielke K, Brecht S, Dorst A and Herdegen T. (1999) Activity and expression of JNK1, p38 and ERK kinases, c-Jun N-terminal phosphorylation, and c-jun promoter binding in the adult rat brain following kainate-induced seizures. Neuroscience. 91(2):471-83.

Mielke K and Herdegen T. (2000) JNK and p38 stresskinases--degenerative effectors of signal-transduction-cascades in the nervous system. Prog Neurobiol. May;61(1):45-60.

Migheli A, Piva R, Atzori C, Troost D and Schiffer D. (1997) c-Jun, JNK/SAPK kinases and transcription factor NF-kappa B are selectively

activated in astrocytes, but not motor neurons, in amyotrophic lateral sclerosis. J Neuropathol Exp Neurol. Dec;56(12):1314-22.

Milton NGN. (2001) Phosphorylation of amyloid-β at the serine 26 residue by human cdc2 kinase. Neurochemistry 12, 3839-3844.

Minghetti L and Levi G. (1998) Microglia as effector cells in brain damage and repair: focus on prostanoids and nitric oxide. Prog Neurobiol 54, 99-125.

Minogue AM, Schmid AW, Fogarty MP, Moore AC, Campbell VA, Herron CE and Lynch MA. (2003) Activation of the JNK signalling cascade mediates the e.ect of Ab on LTP and cell death in hippocampus: a role for interleukin-1b. J. Biol. Chem. 278 (30), 27971 - 27980.

Miyasaka T, Miyasaka J and Saltiel AR. (1990) Okadaic acid stimulates the activity of microtubule associated protein kinase in PC-12 pheochromocytoma cells. Biochem. Biophys. Res. Comm. 168, 1237-1243.

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

Morris RGM, Moser EI, Riedel G, Martin SJ, Sandin J, Day M and O'Carroll C. (2003). Elements of a neurobiological theory of the hippocampus: the role of activity-dependent synaptic plasticity in memory. Phil Trans R Soc Lond B Biol Sci, 29;358, 773-86

Mucke L, Masliah E, Yu GQ, Mallory M, Rockenstein EM, Tatsuno G, Hu K, Kholodenko D, Johnson-Wood K, McConlogue L. (2000) High-level neuronal expression of A β1–42 in wild-type human amyloid protein precursor transgenic mice: synaptotoxicity without plaque formation. J. Neurosci. 20 pp. 4050–4058.

Munoz JP, Alvarez A and Maccioni RB. (2000) Increase in the expression of the neuronal cyclin-dependent protein kinase cdk-5 during differentiation of N2A neuroblastoma cells. Neuroreport. Aug 21;11(12):2733-8.

Nakagami Y and Oda T. (2002) Glutamate exacerbates amyloid β 1–42-induced impairment of long-term potentiation in rat hippocampal slices. Jpn J. Pharmacol. 88, 223–226.

Nakanishi S. (1992) Molecular diversity of glutamate receptors and implications for brain function. Science m: 597-603.

Naslund J, Haroutunian V, Mohs R, Davis KL, Davies P, Greengard P and Buxbaum JD. (2000) Correlation between elevated levels of amyloid β-peptide in the brain and cognitive decline. JAMA 283, 1571-7.

Nordberg A, Romanelli L, Sundwall A, Bianchi C and Beani L. (1989) Effect of acute and subchronic nicotine treatment on cortical acetylcholine release and on nicotinic receptors in rats and guinea pigs. Br. J. Pharmacol. 98, 71–78.

Nordberg A. (2001) Nicotinic receptor abnormalities of Alzheimer's disease: therapeutic implications. Biol. Psychiatry 49, 200–210.

Nguyen MD, Julien JP and Rivest S. (2002) Innate immunity: the missing link in neuroprotection and neurodegeneration. Nat. Rev. Neurosci 3, 216-227.

Oda T, Wals P, Osterburg HH, Johnson SA, Pasinetti GM, Morgan TE, Rozovsky I, Stine WB, Snyder SW and Holzman TF. (1995) Clusterin (apoJ) alters the aggregation of amyloid beta-peptide (A beta 1-42) and forms slowly sedimenting A beta complexes that cause oxidative stress. Exp Neurol. 136(1):22-31.

O'Hara PJ, Sheppard PO, Thogersen H, Venezia D, Haldeman BA, McGrane V, Houamed KM, Thomsen C, Gilbert TL and Mulvihill ER. (1993) The ligand-binding domain in metabotropic glutamate receptors is related to bacterial periplasmic binding proteins. Neuron. Jul;11(1):41-52.

Okamoto N, Hori S, Akazawa C, Hayashi Y, Shigemoto R, Mizuno N and Nakanishi S. (1994) Molecular characterization of a new metabotropic glutamate receptor mGluR7 coupled to inhibitory cyclic AMP signal transduction. J Biol Chem. Jan 14;269(2):1231-6.

Ono K and Han J (2000) The p38 signal transduction pathway. Activation and function. Cellular signalling 12, 1-13.

Oo TF, Henchcliffe C, James D and Burke RE. (1999) Expression of c-fos, c-jun, and c-jun N-terminal kinase (JNK) in a developmental model

of induced apoptotic death in neurons of the substantia nigra. J Neurochem. Feb;72(2):557-64.

Paglini G, Pigino G, Kunda P, Morfini G, Maccioni R, Quiroga S, Ferreira A and Caceres A. (1998) Evidence for the participation of the neuron-specific CDK5 activator P35 during laminin-enhanced axonal growth. J Neurosci. Dec 1;18(23):9858-69.

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

Parpura-Gill A, Beitz D and Uemura E. (1997) The inhibitory effects of betaamyloid on glutamate and glucose uptakes by cultured astrocytes. Brain Res; 754:65.

Paterson D and Nordberg A. (2000) Neuronal nicotinic receptors in the human brain. Prog. Neurobiol. 61, 75–111.

Patrick GN, Zukerberg L, Nikolic M, de la Monte S, Dikkes P and Tsai LH (1999) Conversion of p35 to p25 deregulates Cdk5 activity and promotes neurodegeneration. Nature 402, 615-22.

Paul A, Wilson S, Belham CM, Robinson CJM, Scott PH, Gould GW and Plevin R. (1997) Stress-activated kinases: activation, regulation and function. Cell Sigmal. 9, 403-410.

Pedersen WA and Blusztajn JK. (1997) Characterization of the acetylcholine-reducing effect of the amyloid beta-peptide in mouse SN56 cells. Neurosci. Lett. 239, 77–80.

Pedersen WA, Kloczewiak MA and Blusztajn JK. (1996) Amyloid beta-protein reduces acetylcholine synthesis in a cell line derived from cholinergic neurons of the basal forebrain. Proc. Natl. Acad. Sci. U.S.A. 93, 8068–8071.

Peltekova V, Han G, Soleymanlou N and Hampson DR. (2000) Constraints on proper folding of the amino terminal domains of group III metabotropic glutamate receptors. Brain Res Mol Brain Res. Mar 10;76(1):180-90.

Perkinton MS, Sihra TS and Williams RJ. (1999) Ca(2+)- permeable AMPA receptors induce phosphorylation of cAMP response element-binding protein through a phosphatidylinositol 3-kinase-dependent stimulation of the mitogen-activated protein kinase signaling cascade in neurons. J. Neurosci. 19, 5861-5874.

Perry G, Roder H, Nunomura A, Takeda A, Friedlich AL, Zhu X, Raina AK, Holbrook N, Siedlak SL, Harris PLR and Smith MA (1999) Activation of neuronal extracellular receptor kinase (ERK) in Alzheimers disease links oxidative stress to abnormal phosphorylation. NeuroReport 10, 2411-2415.

Perry RJ and Hodges JR. (1999) Attention and executive deficits in Alzheimer's disease. A critical review. Brain. Mar;122 (Pt 3):383-404.

Pettit DL, Shao Z and Yakel JL. (2001) Beta-amyloid(1–42) peptide directly modulates nicotinic receptors in the rat hippocampal slice. J. Neurosci. 21, RC120.

Pigino G, Paglini G, Ulloa L, Avila J and Caceres A. (1997) Analysis of the expression, distribution and function of cyclin dependent kinase 5 (cdk5) in developing cerebellar macroneurons. J Cell Sci. Jan;110 :257-70.

Pike CJ, Burdick D, Walencewicz AJ, Glabe CG and Cotman CW. (1993) Neurodegeneration induced by beta-amyloid peptides in vitro: the role of peptide assembly state. J Neurosci. Apr;13(4):1676-87.

Pin JP and Duvoisin R. (1995) The metabotropic glutamate receptors: Structure and functions. Neuropharmacol. 34: l-26.

Pitschke M, Prior R, Haupt M and Riesner D. (1998) Detection of single amyloid beta-protein aggregates in the cerebrospinal fluid of Alzheimer's patients by fluorescence correlation spectroscopy. Nat. Med. 4 pp. 832–834.

Podlisny MB, Ostaszewski BL, Squazzo SL, Koo EH, Rydell RE, Teplow DB and Selkoe DJ. (1995) Aggregation of secreted amyloid β-protein into sodium dodecyl sulfate-stable oligomers in cell culture. J. Biol. Chem. 270 pp. 9564–9570.

Pyo H, Jou I, Jung S, Hong S and Joe EH. (1998) Mitogen-activated protein kinases activated by lipopolysaccharide and β-amyloid in cultured rat microglia. NeuroReport 9, 871-874.

Qin L, Liu Y, Cooper C, Liu B, Wilson B and Hong JS. (2002) Microglia enhance β-amyloid peptide-induced toxicity in cortical and mesencephalic neurons by producing reactive oxygen species. J. Neurochem 83, 973-983.

Quirion R. (1993) Cholinergic markers in Alzheimer disease and the autoregulation of acetylcholine release. J. Psychiatry Neurosci. 18, 226–234.

Quirion R, Richard J and Wilson A. (1994) Muscarinic and nicotinic modulation of cortical acetylcholine release monitored by in vivo microdialysis in freely moving adult rats. Synapse 17, 92–100.

Rapoport M and Ferreira A. (2000) PD98059 prevents neurite degeneration induced by fibrillar beta-amyloid in mature hippocampal neurons. J Neurochem. Jan;74(1):125-33.

Raymond CR, Ireland DR and Abraham WC (2003) NMDA receptor regulation by amyloid-β does not account for its inhibition of LTP in rat hippocampus. Brain Research 968, 263-272.

Reymann KG and Matthies H. (1989) 2-Amino-4-phosphonobutyrate selectively eliminates late phases of long-term potentiation in the rat hippocampus. Neurosci. Lett. B: 166-171.

Reynolds CH, Utton MA, Gibb GM, Yates A and Anderton BH. (1997) Stress-activated protein kinase/c-jun N-terminal kinase phosphorylates tau protein. J Neurochem. Apr;68(4):1736-44.

Robakis NK, Wisniewski HM, Jenkins EC, Devine-Gage EA, Houck GE, Yao XL, Ramakrishna N, Wolfe G, Silverman WP and Brown WT. (1987) Chromosome 21q21 sublocalisation of gene encoding beta-amyloid peptide in cerebral vessels and neuritic (senile) plaques of people with Alzheimer disease and Down syndrome. Lancet. 14;1(8529):384-5.

Robertson ED, English JD, Adams JP, Selcher JC, Kondratick C and Sweatt JD (1999) The mitogen-activated ptotein kinase cascade couples PKA and PKC to cAMP response element binding protein phosphorylation in area CA1 of hippocampus. J. Neurosci. 19, 4337-4348.

Robinson MJ and Cobb MH. (1997) Mitogen-activated protein kinase pathways. Curr Opin Cell Biol. Apr;9(2):180-6.

Roher AE, Chaney MO, Kuo YM, Webster SD, Stine WB, Haverkamp LJ, Woods AS, Cotter RJ, Tuohy JM, Krafft GA, Bonnell BS, Emmerling MR. (1996) Morphology and toxicity of Abeta-(1-42) dimer derived from neuritic and vascular amyloid deposits of Alzheimer's disease. J. Biol. Chem. 271, 20631-20635.

Rosenblum K, Futter M, Jones M, Hulme EC and Bliss TV. (2000) ERKI/II Regulation by the Muscarinic Acetylcholine Receptors in Neurons. J. Neurosci. 20, 977-985.

Rosenblum K, Futter M, Voss K, Erent M, Skehel PA, French P, Obosi L, Jones MW and Bliss TV. (2002) The role of extracellular regulated kinases I/II in late-phase long-term potentiation. J Neurosci. Jul 1;22(13):5432-41.

Rush AM, Wu J, Rowan MJ and Anwyl R. (2002) Group I metabotropic glutamate receptor (mGluR)-dependent long-term depression mediated via p38 mitogen-activated protein kinase is inhibited by previous high-frequency stimulation and activation of mGluRs and protein kinase C in the rat dentate gyrus in vitro. J Neurosci 22, 6121-8.

Saez JC, Spray DC and Hertzberg EL. (1990) Gap junctions: biochemical properties and functional regulation under physiological and toxicological conditions. In Vitro Toxicol;3:69.

Sala R, Viegi A, Rossi FM, Pizzorusso T, Bonanno G, Raiteri M and Maffei L. 1(998) Nerve growth factor and brain-derived neurotrophic factor increase neurotransmitter release in the rat visual cortex. Eur. J. Neurosci. 10, 2185–2191.

Sanchez, Mejia RO, Ona VO, Li M and Friedlander RM (2001) Minocycline reduces traumatic brain injury-mediated caspase-1 activation, tissue damage and neurological dysfunction. Neurosurgery 48, 1393-1401.

Sato N, Kamino K, Tateishi K, Satoh T, Nishiwaki Y, Yoshiiwa A, Miki T and Ogihara T. (1997) Elevated amyloid beta protein(1-40) level induces CREB phosphorylation at serine-133 via p44/42 MAP kinase (Erk1/2)-dependent pathway in rat pheochromocytoma PC12 cells. Biochem Biophys Res Commun. Mar 27;232(3):637-42.

Satoh Y, Hirakura Y, Shibayama S, Hirashima N, Suzuki T and Kirino Y. (2001) Beta-amyloid peptides inhibit acetylcholine release from

cholinergic pre-synaptic nerve endings isolated from an electric ray. Neurosci. Lett. 302, 97–100.

Savage MJ, Guo YG, Ciallella JR, Flood DG and Scott RW (2002). Activation of c-Jun N-terminal kinase and p38 in an Alzheimers disease model is associated with amyloid deposition. J. Neuroscience 22, 3376-3385.

Scheuner D, Eckman C, Jensen M, Song X, Citron M, Suzuki N, Bird TD, Hardy J, Hutton M, Kukull W, Larson E, Levy-Lahad E, Viitanen M, Peskind E, Poorkaj P, Schellenberg G, Tanzi R, Wasco W, Lannfelt L, Selkoe D and Younkin S. (1996) Secreted amyloid protein similar to that in the senile plaques of Alzheimers disease is increased in vivo by the presenilin 1 and 2 and APP mutations linked to familial Alzheimers disease. Nat Med 2, 864-870.

Schweers O, Mandelkow EM, Biernat J and Mandelkow E. (1995) Oxidation of cysteine-322 in the repeat domain of microtubule-associated protein tau controls the in vitro assembly of paired helical filaments. Proc Natl Acad Sci U S A. Aug 29;92(18):8463-7.

Seabrook GR, Smith DW, Bowery BJ, Easter A, Reynolds T, Fitzjohn SM, Morton RA, Zheng H, Dawson GR, Sirinathsinghji DJS, Davies CH, Collingridge GL and Hill RG (1999) Mechanisms contributing to the deficits in hippocampal synaptic plasticity in mice lacking amyloid precursor protein. Neuropharmacology 38, 349-359.

Selkoe DJ, Abraham CR, Podlisny MB and Duffy LK. (1986) Isolation of low-molecular-weight proteins from amyloid plaque fibers in Alzheimer's disease. J Neurochem. Jun;46(6):1820-34.

Selkoe DJ. (1997) Alzheimer's disease: genotypes, phenotypes, and treatments. Science 275, 630–631.

Selkoe DJ (2000) Toward a comprehensive theory for Alzheimer's disease. Hypothesis: Alzheimer's disease is caused by the cerebral accumulation and cytotoxicity of amyloid beta-protein. Ann N Y Acad Sci. 924:17-25. Review.

Selkoe DJ. (2001) Alzheimer's disease: genes, proteins, and therapy. Physiol Rev. 81(2):741-66.

Selkoe DJ. (2002) Alzheimers disease is a synaptic failure. Science 298, 789-791.

Shoji M, Iwakami N, Takeuchi S, Waragai M, Suzuki M, Kanazawa I, Lippa CF, Ono S and Okazawa H (2000). JNK activation is associated with intracellular □ β-amyloid accumulation. Brain Res. Mol. Brain Res. 85, 221-233.

Shigemoto R, Kinoshita A, Wada E, Nomura S, Ohishi H, Takada M, Flor PJ, Neki A, Abe T, Nakanishi S and Mizuno N. (1997) Differential presynaptic localization of metabotropic glutamate receptor subtypes in the rat hippocampus. J Neurosci. Oct 1;17(19):7503-22.

Sisodia SS, Koo EH, Beyreuther K, Unterbeck A, Price DL. (1990) Evidence that beta-amyloid protein in Alzheimer's disease is not derived by normal processing. Science. 27;248(4954):492-5.

Small DH, Mok SS and Bornstein JC. (2001) Alzheimer's disease and A β toxicity: from top to bottom. Nat Rev Neurosci 8, 595-8.

Smith CJ, Perry EK, Perry RH, Fairbairn AF and Birdsall NJ.(1987) Guanine nucleotide modulation of muscarinic cholinergic receptor binding in post-mortem human brain—a preliminary study in Alzheimer's disease. Neurosci. Lett. 82, 227–232.

Smith MA, Richey-Harris, PL, Sayre LM, Beckman JS and Perry G (1997) Widespread peroxynitrite-mediated damage in Alzheimer's disease.J. Neurosci 17, 2653-2657.

Smith MA. (1998) Alzheimer disease. Int Rev Neurobiol.;42:1-54.

Smith MA, Sayre LM, Anderson VE, Harris PL, Beal MF, Kowall N and Perry G. (1998) Cytochemical demonstration of oxidative damage in Alzheimer disease by immunochemical enhancement of the carbonyl reaction with 2,4-dinitrophenylhydrazine. J Histochem Cytochem;46:731.

Smith M, Harris P, Sayre L and Perry G. (1997) Iron accumulation in Alzheimer disease is a source of redox-generated free radicals. Proc Natl Acad Sci USA;94:9866.

Sorbi S, Bird ED and Blass JP. (1983) Decreased pyruvate dehydrogenase complex activity in Huntington and Alzheimer brain. Ann. Neurol. 13, 72–78.

Soto C, Castano EM, Frangione B and Inestrosa NC. (1995) The alpha-helical to beta-strand transition in the amino-terminal fragment of the amyloid beta-peptide modulates amyloid formation. J Biol Chem. 17;270(7):3063-7.

Stephan A, Laroche S and Davis S. (2001) Generation of aggregated β-amyloid in the rat hippocampus impairs synaptic transmission and plasticity and causes memory deficits. J. Neurosci 21, 5703-5714.

Su JH, Deng G and Cotman CW. (1997) Neuronal DNA damage precedes tangle formation and is associated with up-regulation of nitrotyrosine in Alzheimer's disease brain. Brain Res;774:193.

Sugden PH and Clerk A. (1997) Regulation of the ERK subgroup of MAP kinase cascades through G protein-coupled receptors. Cell Signal 9:337–351.

Sweatt JD. (2001) The neuronal MAP kinase cascade: a biochemical signal integration system subserving synaptic plasticity and memory. J. Neurochemistry 76, 1-10.

Stephan A, Laroche S and Davis S. (2001) Generation of aggregated β-amyloid in the rat hippocampus impairs synaptic transmission and plasticity and causes memory deficits. J. Neurosci 21, 5703-5714.

Suzdak PD. Thomsen C. Mulvihill E. and Krisensen P. (1994) Molecular cloning, expression, and characterization of metabotropic glutamate receptor subtypes. In The metabotropic glutamate receptors, PJ Corm and J Plate1 (Eds.), pp. l-30.

Takashima A, Noguchi K, Sato K, Hoshino T and Imahori K. (1993) Tau protein kinase I is essential for amyloid beta-protein-induced neurotoxicity. Proc Natl Acad Sci U S A. Aug 15;90(16):7789-93.

Tan J, Town T, Paris D, Mori T, Suo Z, Crawford F, Mattson MP, Flavell RA and Mullan M. (1999) Microglial activation resulting from CD40-CD40L interaction after beta-amyloid stimulation. Science 286, 2352-2355.

Terzi E, Holzemann G and Seelig J. (1995) Self-association of beta-amyloid peptide (1-40) in solution and binding to lipid membranes. J Mol BiolOct 6;252(5):633-42.

Terry RD, Masliah E, Salmon DP, Butters N, DeTeresa R, Hill R, Hansen LA and Katzman R. (1991) Physical basis of cognitive alterations in Alzheimers didease: synapse loss is the major correlate of cognitive impairment. Ann Neurol. 30, 572-580 (1991).

Tikka T, Fiebich B, Goldsteins G, Keinanen R and Koistinaho J (2001) Minocycline, a tetracycline derivative, is neuroprotective against excitiotoxicity by inhibiting activating and proliferation of microglia. J. Neuroscience 21, 2580-2588.

Tokuda T, Fukushima T, Ikeda S, Sekijama Y, Shoji S, Yanagisawa N and Tamaoka A (1997) Plasma levels of amyloid beta protein A β 1-40 and A β1-42 are elevated in Downs syndrome. Ann Neurol 4, 271-273.

Tong L, Thornton PL, Balazs R. and Cotman CW. (2001) β-Amyloid-(1–42) impairs activity-dependent cAMP-response element-binding protein signaling in neurons at concentrations in which cell suvival is not compromised. J. Biol. Chem. 276, 17 301–17 306.

Toni N, Buchs PA, Nikonenko I, Bron CR and Muller D. (1999) LTP promotes formation of multiple spine synapses between a single axon terminal and a dendrite. Nature 402: 421-425 (1999).

Town T, Zolton J, Shaffner R, Schnell B, Crescentini R, Wu Y, Zeng J, DelleDonne A, Obregon D, Tan J and Mullan M. (2002) p35/Cdk5 pathway mediates soluble amyloid-β peptide-induced tau phosphorylation in vitro. J Neurosci Res 69, 362-72

Trotti D, Rossi D, Gjesdal O, Levy LM, Racagni G, Danbolt NC and Volterra A. (1996) Peroxynitrite inhibits glutamate transporter subtypes. J Biol Chem;271:5976.

Troy CM, Rabaccji SA, Xu Z, Maraney AC, Connors TJ, Shelanski ML and Greene LA. (2001) β-amyloid-induced neuronal apoptosis requires c-Jun N-terminal kinase activation. J. Neurochem 77, 157-164.

Vaucher E, Aumont N, Pearson D, Rowe W, Poirier J and Kar S. (2001) Amyloid beta-peptide levels and its effects on hippocampal acetylcholine

release in aged, cognitively-impaired and –unimpaired rats. J. Chem. Neuroanat. 21, 323–329.

Vitolo OV, Sant'Angelo A, Costanzo V, Battaglia F, Arancio O and Shelanski M. (2002) Amyloid β-peptide inhibition of the PKA/CREB pathway and long-term potentiation: reversibility by drugs that enhance cAMP signaling. Proc Natl Acad Sci U S A 99, 13217-21.

Volterra A, Trotti D, Tromba C, Floridi S and Racagni G. (1994) Glutamate uptake inhibition by oxygen free radicals in rat cortical astrocytes. J Neurosci;14:2924.

Wallace MN, Geddes, JG, Farquar DA and Masson MR (1997). Nitric oxide synthase in reactive astrocytes adjacent to beta-amyloid. Exp. Neurol. 144, 266-272.

Walsh D M, Hartley DM, Kusumoto Y, Fezoui Y, Condron MM, Lomakin A, Benedek, GB, Selkoe DJ and Teplow DB. (1999) Amyloid beta-protein fibrillogenesis. Structure and biological activity of protofibrillar intermediates. J. Biol. Chem. 274, pp. 25945–25952.

Walsh DM, Lomakin A, Benedek GB, Condron MM and Teplow DB. (1997) Amyloid β-protein fibrillogenesis. Detection of a protofibrillar intermediate. J Biol Chem. 272, 22364-72

Walsh DM, Tseng BP, Rydel RE, Podlisny MB and Selkoe DJ (2000) detection of intracellular oligomers of amyoid □ β-protein in cells derived from human brain. Biochemistry 39, 10831-10839.

Walsh, DM, Klyubuin I, Fadeeva, J, Cullen WK, Anwyl R, Wolfe MS, Rowan MJ and Selkoe DJ (2002) Naturally secreted oligomers of the Alzheimer amyloid β-protein potently inhibit long-term potentiation in vivo. Nature 416, 535-539.

Wang HY, Wild KD, Shank RP and Lee DH. (1999) Galanin inhibits acetylcholine release from rat cerebral cortex via a pertussis toxin-sensitive G(i)protein. Neuropeptides 33, 197–205.

Wang HY, Lee DH, D'Andrea MR, Peterson PA, Shank RP and Reitz AB. (2000) Beta-amyloid(1–42) binds to alpha7 nicotinic acetylcholine receptor with high affinity. Implications for Alzheimer's disease pathology. J. Biol. Chem. 275, 5626–5632.

Wang HY, Lee DH, Davis CB, Shank RP. (2000) Amyloid peptide Abeta(1–42) binds selectively and with picomolar affinity to alpha7 nicotinic acetylcholine receptors. J. Neurochem. 75, 1155–1161.

Wang HW, Pasternak JF, Kuo H, Ristic H, Lambert MP, Chromy B, Violoa KL, Klein WL, Stine WB, Krafft GA and Trommer BL (2002) Soluble oligomers of β-amyloid (1-42) inhibit long-term depression but not long-term depression in the dentate gyrus. Brain research 924, 133-140.

Wang J, Dickson DW, Trojanowski JQ and Lee VM. (1999) The levels of soluble versus insoluble brain Abeta distinguish Alzheimer's disease from normal and pathologic aging. Exp. Neurol. 158, 328–337

Wang Q, Walsh DM, Rowan MJ, Selkoe DJ and Anwyl R. (2004) Block of LTP by naturally secreted and synthetic amyloid β-peptide in hippocampal slices is mediated via activation of the kinases JNK, Cdk5 and p38 MAPK, and mGluR5. J. Neuroscience 24, 3370-3378.

Warpman U, Alafuzoff I and Nordberg A. (1993) Coupling of muscarinic receptors to GTP proteins in post-mortem human brain—alterations in Alzheimer's disease. Neurosci. Lett. 150, 39–43.

Wei W, Wang X and Kusiak JW. (2002) Signaling events in amyloid beta-peptide-induced neuronal death and insulin-like growth factor I protection. J Biol Chem. May 17;277(20):17649-56. Epub 2002 Mar 06.

Weidemann A, Kong G, Bunke D, Fischer P, Salbaum JM, Masters CL, Beyreuther K and Identification. (1989) Biogenesis, and localization of precursors of Alzheimer's disease A4 amyloid protein. Cell. 7;57(1):115-26.

Winder DG, Martin K, Muzzo I, Rohrer D. Chruscinski A, Kobilka B and Kandel E. (1999) ERK plays a novel regulatory role in the induction of LTP by theta frequency stimulation and its regulation by beta-adrenergic receptors in CA1 pyramidal cells. Neuron 24, 715-726.

Wisniewski T, Ghiso J, and Frangione B. (1991) Peptides homologous to the amyloid protein of Alzheimer's disease containing a glutamine for glutamic acid substitution have accelerated amyloid fibril formation . Biochem . Biophys. Res. Commun. 179, 1247-1254 .

Wu J, Anwyl R and Rowan MJ. (1995) β-amyloid-(1–40) increases long-

term potentiation in rat hippocampus in vitro. Eur. J. Pharmacol. 284, R1–R3.

Wu J, Anwyl R and Rowan MJ. (1995) β-Amyloid selectively augments NMDA receptor-mediated synaptic transmission in rat hippocampus. NeuroReport 6, 2409–2413.

Wu SP, Lu KT, Chang WC and Gean PW. (1999) Involvement of mitogen-activated protein kinase in hippocampal long-term potentiation. J. Biomed Sci. 6, 409-417.

Wu J, Rowan MJ and Anwyl R. (2004) An NMDAR-independent LTP mediated by group II metabotropic glutamate receptors and p42/44 MAP kinase in the dentate gyrus in vitro. Neuropharmacology 46, 311-31.

Xia Z, Dickens M, Raingeaud J, Davis RJ and Greenberg ME. (1995) Opposing effects of ERK and JNK-p38 MAP kinases on apoptosis. Science. Nov 24;270(5240):1326-31.

Xia W, Zhang J, Kholodenko D, Citron M, Podlisny MB, Teplow DB, Haass C, Seubert P, Koo EH and Selkoe DJ (1997) Enhanced production and oligomerization of the 42-residue amyloid β-protein by Chinese hamster ovary cells stably expressing mutant presenilins. J. Biol. Chem. 272, 7977–7982

Yan SD, Chen X, Fu J, Zhu H, Roher A, Slattery T, Morser J, Nawroth P, Stern D and Schmidt AM (1996) RAGE and amyloid-beta peptide neurotoxicity in Alzheimers disease. J. Biol. Chem 274, 15493-15499.

Yan SD, Chen X, Fu J, Chen M, Zhu H, Roher A, Slattery T, Zhao L, Nagashima M, Morser J, Migheli A, Nawroth P, Stern D and Schmidt AM. (1996) RAGE and amyloid-β peptide neurotoxicity in Alzheimer's disease. Nature;382:685.

Yang D, Tournier C, Wysk M, Lu HT, Xu J, Davis RJ and Flavell RA. (1997) Targeted disruption of the MKK4 gene causes embryonic death, inhibition of c-Jun NH2-terminal kinase activation, and defects in AP-1 transcriptional activity. Proc Natl Acad Sci U S A. Apr 1;94(7):3004-9.

Yasojima K, Schwab C, McGeer EG and McGeer PL. (1999) Upregulated production and activation of the complement system in Alzheimer disease brain. Am J Pathol;154:927.

Ye L and Qiao JT. (1999) Suppressive action produced by beta-amyloid peptide fragment 31–35 on long-term potentiation in rat hippocampus is *N*-methyl-d-aspartate receptor-independent: it's offset by (-)huperzine A. Neurosci. Lett. 275, 187–190.

Yrjanheikk J, Keinnanen R, Pellikka M, Hokfelt T and Koistinaho J (1998). Tetracyclines inhibit microglial activation and are neuroprotective in global brain ischaemia. Proc. Natl. Acad. Sci 95, 15769-15774.

Zagorski M. and Barrow C. (1992) NMR studies of amyloid beta-peptides : proton assignments, secondary structure, and mechanism of an a-helix to beta-sheet conversion for a homologous 28-residue, N-terminal fragment . Biochemistry 31, 5621-5631 .

Zhu S, Stavrovskaya IG, Drozda M, Kim BY, Ona V, Li M, Sarang S, Liu AS, Hartley DM, Wu du C, Gullans S, Ferrante RJ, Przedborski S, Kristal BS and Friedlander RM. (2002) Minocycline inhibits cytochrome c release and delays progression of amyotrophic lateral sclerosis in mice. Nature 417, 74-78.

Zhu X, Rottkamp CA, Boux H , Takeda A, Shimohama S, Perry G and Smith MA (2000) Activation of p38 MAP kinase links tau phosphorylation, oxidative stress and cell cycle-related events in Alzheimers disease. J. Neuropathol Exp Neurol 59, 880-888.

Zhu X, Raina AK,Rottkamp CA, Aliev G, Perry G and Smith MA (2001) Activation and redistribution of c-Jun N-terminal kinase/stress activated protein kinase in degenerating neurons in Alzheimers disease. J. Neurochem 76, 435-441.

Zhu X, Rottkamp CA, Hartzler A, Sun Z, Takeda A, Boux H, Shimohama S, Perry G and Smith MA (2001) Activation of MKK6, an upstream activator of p38, in Alzheimers disease. J. Neurochem 79, 311-318.