Main S & T Results of MEMOLOAD

The main results are presented below divided by work packages. As the logic flows from compound synthesis to evaluation of its effects, this reports starts with WP6.

WP6

A. Objective and tasks

WP6 has two main objectives: (1) to produce and characterize the conformation of synthetic Aβ peptides to mimic pathology in AD and (2) to design, synthesize and structurally optimize novel peptidomimetics, which are capable of blocking the toxic effects of Aβ aggregates.

The main objectives are divided into the following **tasks** :
D6.1 Structural characterization and standardized preparation of synthetic Abeta species
D6.2 Production of synthetic oligomeric and fibrillar Abeta for the consortium needs
D6.3 Characterization of natural and synthetic Abeta species from slice recordings
D6.4 Computer aided design of novel anti-Abeta peptidomimetics
D6.5 Optimization of structure and validation of novel anti-Abeta peptidomimetics
D6.6 Production of anti-oligomer and anti-fibril peptide for the consortium needs
D6.7 Pharmacokinetic studies on novel peptidomimetics using tritium labeling

D6.1. Structural characterization and standardized preparation of synthetic Abeta species

The synthesis of A beta peptides has been difficult, owing to their hydrophobic character, poor solubility and high tendency for aggregation. Recently an isopeptide precursor (iso-A beta(1-42)) was synthesized by Fmoc-chemistry and transformed at neutral pH to A beta(1-42) by O-->N acyl migration in a short period of time. We prepared the same precursor peptide using Boc-chemistry and studied the transformation to A beta(1-42) by acyl migration. The peptide conformation and aggregation processes were studied by several methods (circular dichroism, atomic force and transmission electron microscopy, dynamic light scattering). The biological activity of the synthetic A beta(1-42) was measured by ex vivo (long-term potentiation studies in rat hippocampal slices) and in vivo experiments (spatial learning of rats). It was proven that O-->N acyl migration of the precursor isopeptide results in a water soluble oligomeric mixture of neurotoxic A beta(1-42). These oligomers are formed in situ just before the biological experiments and their aggregation grade could be standardized.

Published in:
Bozso Z, Penke B, Simon D, Laczkó I, Juhász G, Szegedi V, Kasza A, Soós K, Hetényi A, Wéber E, Tóháti H, Csete M, Zarándi M, Fülöp L. Controlled in situ preparation of A beta(1-42) oligomers from the isopeptide "iso-A beta(1-42)", physicochemical and biological characterization.
Peptides. 2010 Feb;31(2):248-56

D6.3 Characterization of natural and synthetic Abeta species from slice recordings

First we developed a method to determine toxicity of the synthesized Abeta species

The widely used MTT-assay in cell lines or primary cell cultures could be insensitive against Aβ-peptides. We describe here an easy and relevant method for testing Aβ 1-42 toxicity on acute hippocampal slices derived from rat. Brain slice viability in different conditions was measured using MTT and LDH assays. The concomitant use of these two assays can give detailed and relevant results on the toxic effect of Aβ 1-42 in oxygen-glucose deprived (OGD) acute brain slice model. Both assays are capable of quantifying tissue viability by measuring optical density (OD). We found that simultaneous application of OGD and Aβ 1-42 treatment induced a more intensive decrease in hippocampal slice viability than their separate effects. The use of MTT and LDH assay for quantifying brain slice viability proved to be an easy ex vivo method for investigating Aβ toxicity. Testing brain slices is more relevant in Alzheimer's Disease research than using in vitro cell cultures, due to maintenance of the three dimensional cellular network, the cell variability and intact cell connections.

Published in:
Mozes E, Hunya A, Posa A, Penke B, Datki Z. A novel method for the rapid determination of beta-amyloid toxicity on acute hippocampal slices using MTT and LDH assays. Brain Res Bull. 2012 Apr 10;87(6):521-5.

Then we characterized the effects of Abeta species in two electrophysiological models: the stardard hippocampal slice LTP and in the in vivo recording of hippocampal pyramidal cell firings in rats in responsed to local iontophoretic application.

Converging lines of evidence support the notion that AD begins with subtle alterations in synaptic efficacy, prior to the occurrence of extensive neuronal degeneration. Recently, however, a shared or overlapping pathogenesis for AD and epileptic seizures occurred as aberrant neuronal hyperexcitability, as well as nonconvulsive seizure activity were found in several different APP transgenic mouse lines. This generated a renewed attention to the well-known comorbidity of AD and epilepsy and interest in how Abeta oligomers influence neuronal excitability. In this study therefore, we investigated the effect of various in vitro-aged Abeta(1-42) oligomer solutions on the perforant pathway-evoked field potentials in the ventral hippocampal dentate gyrus in vivo. Firstly, Abeta oligomer solutions (1 microl, 200 microM) which had been aggregated in vitro for 0, 24 or 72h were injected into the hippocampus of urethane-anesthetized rats, in parallel with in vitro physico-chemical characterization of Abeta oligomerization (atomic force microscopy, thioflavin-T fluorescence). We found a marked increase of hippocampal population spike (pSpike) after injection of the 24-h Abeta oligomer solution and a decrease of the pSpike amplitude after injection of the 72-h Abeta oligomer. Since urethane anesthesia affects the properties of hippocampal evoked potentials, we repeated the injection of these two Abeta oligomer solutions in awake, freely moving animals. Evoked responses to perforant pathway stimulation revealed a 70% increase of pSpike amplitude 50 min after the 24-h Abeta oligomer injection and a 55% decrease after the 72-h Abeta oligomer injection. Field potentials, that reflect synaptic potentials, were not affected by the Abeta injection. These results demonstrate that oligomeric Abeta aggregates elicit opposite electrophysiological effects on neuronal excitability which depend on their degree of oligomerization.

Published in:
Orbán G, Völgyi K, Juhász G, Penke B, Kékesi KA, Kardos J, Czurkó A. Different electrophysiological actions of 24- and 72-hour aggregated amyloid-beta oligomers on hippocampal field population spike in both anesthetized and awake rats. Brain Res. 2010 Oct 1;1354:227-35

We have also further characterized the role of NMDA signaling in mediating the Abeta effects.

The aggregated form of amyloid-beta (Abeta) (1-42) has been shown to increase N-methyl-D-aspartic acid (NMDA) evoked neuronal activity in vivo. Here we further characterized this phenomenon by investigating the role of integrin activation and downstream Src kinase activity using in vivo electrophysiology and in vitro intracellular Ca (2+) measurements. Pretreatment of differentiated SH-SY5Y cells with fibrillar Abeta (1-42) markedly enhanced the intracellular calcium increases caused by NMDA receptor (NMDA-R) stimulation. Function blocking antibody against beta1 integrin depressed the facilitatory effects of Abeta (1-42). Similarly, Abeta (1-42) facilitated NMDA-R driven firing of hippocampal neurons in vivo, and this effect was reduced by neutralizing antibody against beta1 integrins. The positive action of Abeta (1-42) on NMDA-R dependent responses was also depressed by an inhibitor known to block Src kinase. These results support the hypothesis that aggregated Abeta (1-42) is recognized by the beta1 subunit containing integrins and may induce a Src kinase dependent NMDA receptor phosphorylation.

Published in:
Uhász GJ, Barkóczi B, Vass G, Datki Z, Hunya A, Fülöp L, Budai D, Penke B, Szegedi V. Fibrillar Abeta (1-42) enhances NMDA receptor sensitivity via the integrin signaling pathway. J Alzheimers Dis. 2010;19(3):1055-67

D6.4 – 6.7 Design, optimization and validation of novel anti-Abeta peptidomimetics

We have designed and synthetized a dozen of different peptidomimetics, of which two were particularly promising:

1A, BAM-9: Phenylopropionyl-DPhe-DPro-δ-amino-valeryl-amide (M_w=492.3)

C. de Soto's peptide (Leucyl-Prolyl-Phenylalanyl-Phenylalanyl-Aspartyl-amide; LPFFDa) was used as lead compound for further design performed by computer simulation methods. The main aim was planning a neuroprotective peptidomimetic compound fully resistant against proteolytic enzymes. Our hypothesis was: Soto's peptide LPFFDa can bind the β-structured part of Aβ1-42 and prevent further aggregation to toxic Aβ-species, but it is not enzyme resistant. Replacing L-amino acids to D-enantiomers renders full resistance against proteolytic enzymes making such peptidomimetics good drug candidates. Therefore D-amino acids were used as the basis in planning further comopounds. Altogether 85 compounds (among them BAM-9) were designed, synthesized and screened by a modified MTT-assay in SH-SY5Y cell cultures (Datki et al., Brain Research Bulletin, 62(3), 223-229, 2003).

1B. Ac-da-orn$_a$: Acetyl-DAsp-DAla-DOrn-amide

The lead compound for further design was the RGD-peptide: Arg-Gly-Asp-amide, a well-know ligand for cell membrane integrins. Aβ1-42 contains a similar sequence (Arg-His-Asp) and binds to integrins. Computer simulation methods were used for design of peptidomimetics containing D-amino acids for reaching full resistance against proteolytic enzymes. This resulted is a cohort of 30 peptidomimetics, one of them being Ac-da-orn$_a$.

For screening, the neuroprotective effect of the novel compounds against Aβ1-42 was measured in rat hippocampal slices using the modified MTT-assay.

The testing of there in vivo effect has been published.

A promising strategy against AD is the application of protective, peptide-based neuroprotective agents that selectively bind to Abeta. We recently described a pentapeptide, LPYFDa, which recognizes Abeta (1-42) and protects neurons against the toxic effects of aggregated Abeta (1-42) both in vitro and in vivo. Our previous work indicated that the in vivo ejection of fibrillar Abeta (1-42) into the hippocampal CA1 region resulted in a massive increase in the NMDA-evoked neuronal firing rate. Our current aim was to study whether intraperitoneally administered LPYFDa is capable of protecting against the synaptotoxic action of fibrillar Abeta (1-42) administered by iontophoresis. Our investigations of the in vivo biodistribution of tritium-labelled LPYFDa and single-unit electrophysiology revealed that LPYFDa readily crosses the blood-brain barrier, and protects the synapses against the excitatory action of fibrillar Abeta (1-42) in a relatively wide temporal window in rat. This pentapeptide may serve as a lead compound for the design of novel drug candidates for the prevention of AD.

Juhász G, Márki A, Vass G, Fülöp L, Budai D, Penke B, Falkay G, Szegedi V. An intraperitoneally administered pentapeptide protects against Abeta (1-42) induced neuronal excitation in vivo. J Alzheimers Dis. 2009;16(1):189-96.

Misfolding, oligomerization, and aggregation of the amyloid-beta (Abeta) peptide is widely recognized as a central event in the pathogenesis of Alzheimer's disease (AD). Recent studies have identified soluble Abeta oligomers as the main pathogenic agents and provided evidence that such oligomeric Abeta aggregates are neurotoxic, disrupt synaptic plasticity, and inhibit long-term potentiation. A promising therapeutic strategy in the battle against AD is the application of short synthetic peptides which are designed to bind to specific Abeta-regions thereby neutralizing or interfering with the devastating properties of oligomeric Abeta species. In the present study, we investigated the neuroprotective properties of the amyloid sequence derived pentapeptide LPYFDa in vitro as well as its memory preserving capacity against Abeta(42)-induced learning deficits in vivo. In vitro we showed that neurons in culture treated with LPYFDa are protected against Abeta (42) -induced cell death. Moreover, in vivo LPYFDa prevented memory impairment tested in a contextual fear conditioning paradigm in mice after bilateral intrahippocampal Abeta (42) injections. We thus showed for the first time that an anti-amyloid peptide like LPYFDa can preserve memory by reverting Abeta (42) oligomer-induced learning deficits.

Granic I, Masman MF, Kees Mulder C, Nijholt IM, Naude PJ, de Haan A, Borbély E, Penke B, Luiten PG, Eisel UL. LPYFDa neutralizes amyloid-beta-induced memory impairment and toxicity. J Alzheimers Dis. 2010;19(3):991-1005

Amyloid fibrils are self-associating filamentous structures deposited in extracellular tissue in various neurodegenerative and protein misfolding disorders. It has been shown that beta-sheet-breaker (BSB) peptides may interfere with amyloid fibril assembly. Although BSB peptides are prospective therapeutic agents in amyloidosis, there is ambiguity about the mechanisms and generality of their action. In the present work we analyzed the effect of the BSB peptide LPFFD on the growth kinetics, morphologic, and mechanical properties of amyloid β25-35 (Aβ25-35) fibrils assembled in an oriented array on mica surface. Aβ25-35 is thought to represent the biologically active, toxic fragment of the full-length Aβ peptide. Growth kinetics and morphologic features were analyzed using in situ atomic force microscopy in the presence of various concentrations of LPFFD. We found that the addition of LPFFD only slightly altered the assembly kinetics of Aβ25-35 fibrils. Already formed fibrils did not disassemble in the presence of high concentrations of LPFFD. The mechanical stability of the fibrils was explored with force spectroscopy methods. The nanomechanical behavior of Aβ25-35 fibrils is characterized by the appearance of force staircases which correspond to the force-driven unzipping and dissociation of several protofilaments. In the presence of LPFFD single-plateau force traces dominated. The effects of LPFFD

on Aβ25-35 fibril assembly and stability suggest that inter-protofilament interactions were slightly weakened. Complete disassembly of fibrils, however,was not observed. Thus, under the conditions explored here, LPFFD may not be considered as a BSB peptide with generalized beta-sheet breaking properties.

Murvai U, Soós K, Penke B, Kellermayer MS.Effect of the beta-sheet-breaker peptide LPFFD on oriented network of amyloid β25-35 fibrils. J Mol Recognit. 2011 May-Jun;24(3):453-60

WP 1

A. Objective and tasks

The main **objective** of this work package is to design and implement novel behavioural protocols suitable for rodent-based studies whose aim is (1) to identify and then characterize early memory loss in models of Alzheimer's Disease (AD), and (2) to use these models to study the impact of potential therapeutics. This divides further into the following **tasks**:

1.1 Development of new memory screening tests appropriate for testing rodent models of AD.
1.2 Memory loss in transgenic mice arising from endogenous Aβ production and from deficiencies in specific signalling molecules.
1.3 Imaging studies to reveal brain areas of task-related activation.
1.4 Memory loss arising from exogenous Aβ administration.
1.5 Testing of novel drug treatments to counteract Aβ effects
1.6 Collection of brain samples from behaviourally tested animals for molecular studies.

D1.1 Development of new memory screening tests appropriate for testing rodent models of AD

Two experiments were conducted to investigate the possibility of faster forgetting by PDAPP mice (a well-established model of Alzheimer's disease as reported by Games and colleagues in an earlier paper). Experiment 1, using mice aged 13-16 mo, confirmed the presence of a deficit in a spatial reference memory task in the water maze by hemizygous PDAPP mice relative to littermate controls. However, after overtraining to a criterion of equivalent navigational performance, a series of memory retention tests revealed faster forgetting in the PDAPP group. Very limited retraining was sufficient to reinstate good memory in both groups, indicating that their faster forgetting may be due to retrieval failure rather than trace decay. In Experiment 2, 6-mo-old PDAPP and controls were required to learn each of a series of spatial locations to criterion with their memory assessed 10 min after learning each location. No memory deficit was apparent in the PDAPP mice initially, but a deficit built up through the series of locations suggestive of increased sensitivity to interference. Faster forgetting and increased interference may each reflect a difficulty in accessing memory traces. This interpretation of one aspect of the cognitive deficit in human mutant APP mice has parallels to deficits observed in patients with Alzheimer's disease, further supporting the validity of transgenic models of the disease.

Daumas S, Sandin J, Chen KS, Kobayashi D, Tulloch J, Martin SJ, Games D, Morris

RG. Faster forgetting contributes to impaired spatial memory in the PDAPP mouse: deficit in memory retrieval associated with increased sensitivity to interference? Learn Mem. 2008 Aug 26;15(9):625-32.

D1.2 Memory loss in transgenic mice arising from endogenous Aβ production and from deficiencies in specific signalling molecules.

We have focused on two groups of modulating signaling molecules.

(a) BDNF

Brain-derived neurotrophic factor (BDNF) plays an important role in neuronal plasticity, learning, and memory. Levels of BDNF and its main receptor TrkB (TrkB.TK) have been reported to be decreased while the levels of the truncated TrkB (TrkB.T1) are increased in Alzheimer's disease. We show here that incubation with amyloid-β increased TrkB.T1 receptor levels and decreased TrkB.TK levels in primary neurons. In vivo, APPswe/PS1dE9 transgenic mice (APdE9) showed an age-dependent relative increase in cortical but not hippocampal TrkB.T1 receptor levels compared with TrkB.TK. To investigate the role of TrkB isoforms in Alzheimer's disease, we crossed AP mice with mice overexpressing the truncated TrkB.T1 receptor (T1) or the full-length TrkB.TK isoform. Overexpression of TrkB.T1 in APdE9 mice exacerbated their spatial memory impairment while the overexpression of TrkB.TK alleviated it. These data suggest that amyloid-β changes the ratio between TrkB isoforms in favor of the dominant-negative TrkB.T1 isoform both in vitro and in vivo and supports the role of BDNF signaling through TrkB in the pathophysiology and cognitive deficits of Alzheimer's disease.

Kemppainen S, Rantamäki T, Jerónimo-Santos A, Lavasseur G, Autio H, Karpova N, Kärkkäinen E, Stavén S, Vicente Miranda H, Outeiro TF, Diógenes MJ, Laroche S, Davis S, Sebastião AM, Castrén E, Tanila H. Impaired TrkB receptor signaling contributes to memory impairment in APP/PS1 mice. Neurobiol Aging. 2012 Jun;33(6):1122.e23-39. doi: 10.1016/j.neurobiolaging.2011.11.006. Epub 2011 Dec 30.

Brain-derived neurotrophic factor (BDNF) importantly regulates learning and memory and supports the survival of injured neurons. Reduced BDNF levels have been detected in the brains of Alzheimer's disease (AD) patients but the exact role of BDNF in the pathophysiology of the disorder remains obscure. We have recently shown that reduced signaling of BDNF receptor TrkB aggravates memory impairment in APPswe/PS1dE9 (APdE9) mice, a model of AD. The present study examined the influence of Bdnf gene deficiency (heterozygous knockout) on spatial learning, spontaneous exploratory activity and motor coordination/balance in middle-aged male and female APdE9 mice. We also studied brain BDNF protein levels in APdE9 mice in different ages showing progressive amyloid pathology. Both APdE9 and Bdnf mutations impaired spatial learning in males and showed a similar trend in females. Importantly, the effect was additive, so that double mutant mice performed the worst. However, APdE9 and Bdnf mutations influenced spontaneous locomotion in contrasting ways, such that locomotor hyperactivity observed in APdE9 mice was normalized by Bdnf deficiency. Obesity associated with Bdnf deficiency did not account for the reduced hyperactivity in double mutant mice. Bdnf deficiency did not alter amyloid plaque formation in APdE9 mice. Before plaque formation (3 months), BDNF protein levels where either reduced (female) or unaltered (male) in the APdE9 mouse cortex. Unexpectedly, this was followed by an age-dependent increase in mature BDNF protein. Bdnf mRNA and phospho-TrkB levels remained unaltered in the cortical tissue samples of middle-aged APdE9 mice.Immunohistological studies revealed increased BDNF immunoreactivity around amyloid plaques indicating that the plaques may

sequester BDNF protein and prevent it from activating TrkB. If similar BDNF accumulation happens in human AD brains, it would suggest that functional BDNF levels in the AD brains are even lower than reported, which could partially contribute to learning and memory problems of AD patients.

Rantamäki T, Kemppainen S, Autio H, Stavén S, Koivisto H, Kojima M, Antila H, Miettinen PO, Kärkkäinen E, Karpova N, Vesa L, Lindemann L, Hoener MC, Tanila H, Castrén E. The impact of Bdnf gene deficiency to the memory impairment and brain pathology of APPswe/PS1dE9 mouse model of Alzheimer's disease. PLoS One. 2013 Jul 3;8(7):e68722. doi: 10.1371/journal.pone.0068722. Print 2013.

(b) Endocannabinoids

The cannabinoid CB1-receptor is among the most abundant G-protein-coupled receptors in the mammalian brain. Whereas post-mortem studies in Alzheimer's disease (AD) brains compared to age-matched controls have reported decreased CB1-receptor binding but no change in their protein levels (immunoreactivity), decreased or increased CB1- receptor protein levels have been reported in APP/PS1 transgenic mice modelling AD. To complete the picture, the present study used functional autoradiography to assess CB1-receptor-dependent G(i) protein activation in the hippocampus, entorhinal cortex and medial frontal cortex of 13- to 14-month-old female APPswe/PS1dE9 transgenic and wild-type littermate control mice. The mouse brains were processed for $[^{35}S]GTP\lambda S$ autoradiography so that brain sections were analysed in pairs of one transgenic and one control mouse brain. The autoradiography protocol was completed for each pair both in the absence and presence of dithiotreitol (DTT) to reveal possible redox-dependent alterations in CB1 receptor function. Five treatments were used: baseline, incubation with 10 μM GTPλS to assess nonspecific binding, and CB1 receptor agonist CP55,940 in three concentrations. By and large we found no statistically significant differences between the APP/PS1 transgenic and control mice in CB1 receptor signalling. The only exception was a modest redox-dependent alteration in entorhinal cortical CB1 receptors between the genotypes. Thus, in accordance with the majority of earlier human AD findings, we did not find evidence for notable changes in the number of functional CB1 receptors in the common APPswe/PS1dE9 mouse model of AD.

Kärkkäinen E, Tanila H, Laitinen JT. Functional autoradiography shows unaltered cannabinoid CB1 receptor signalling in hippocampus and cortex of APP/PS1 transgenic mice. CNS Neurol Disord Drug Targets. 2012 Dec;11(8):1038-44.

Retrograde messengers adjust the precise timing of neurotransmitter release from the presynapse, thus modulating synaptic efficacy and neuronal activity. 2-Arachidonoyl glycerol, an endocannabinoid, is one such messenger produced in the postsynapse that inhibits neurotransmitter release upon activating presynaptic CB(1) cannabinoid receptors. Cognitive decline in Alzheimer's disease is due to synaptic failure in hippocampal neuronal networks. We hypothesized that errant retrograde 2-arachidonoyl glycerol signalling impairs synaptic neurotransmission in Alzheimer's disease. Comparative protein profiling and quantitative morphometry showed that overall CB(1) cannabinoid receptor protein levels in the hippocampi of patients with Alzheimer's disease remain unchanged relative to age-matched controls, and CB(1) cannabinoid receptor-positive presynapses engulf amyloid-β-containing senile plaques. Hippocampal protein concentrations for the sn-1-diacylglycerol lipase α and β isoforms, synthesizing 2-arachidonoyl glycerol, significantly increased in definite Alzheimer's (Braak stage VI), with ectopic sn-1-diacylglycerol lipase β expression found in microglia accumulating near senile plaques and apposing CB(1) cannabinoid receptor-positive presynapses. We found that microglia, expressing two 2-arachidonoyl glycerol-degrading enzymes, serine hydrolase α/β-hydrolase

domain-containing 6 and monoacylglycerol lipase, begin to surround senile plaques in probable Alzheimer's disease (Braak stage III). However, Alzheimer's pathology differentially impacts serine hydrolase α/β-hydrolase domain-containing 6 and monoacylglycerol lipase in hippocampal neurons: serine hydrolase α/β-hydrolase domain-containing 6 expression ceases in neurofibrillary tangle-bearing pyramidal cells. In contrast, pyramidal cells containing hyperphosphorylated tau retain monoacylglycerol lipase expression, although at levels significantly lower than in neurons lacking neurofibrillary pathology. Here, monoacylglycerol lipase accumulates in CB(1) cannabinoid receptor-positive presynapses. Subcellular fractionation revealed impaired monoacylglycerol lipase recruitment to biological membranes in post-mortem Alzheimer's tissues, suggesting that disease progression slows the termination of 2-arachidonoyl glycerol signalling. We have experimentally confirmed that altered 2-arachidonoyl glycerol signalling could contribute to synapse silencing in Alzheimer's disease by demonstrating significantly prolonged depolarization-induced suppression of inhibition when superfusing mouse hippocampi with amyloid-β. We propose that the temporal dynamics and cellular specificity of molecular rearrangements impairing 2-arachidonoyl glycerol availability and actions may differ from those of anandamide. Thus, enhanced endocannabinoid signalling, particularly around senile plaques, can exacerbate synaptic failure in Alzheimer's disease.

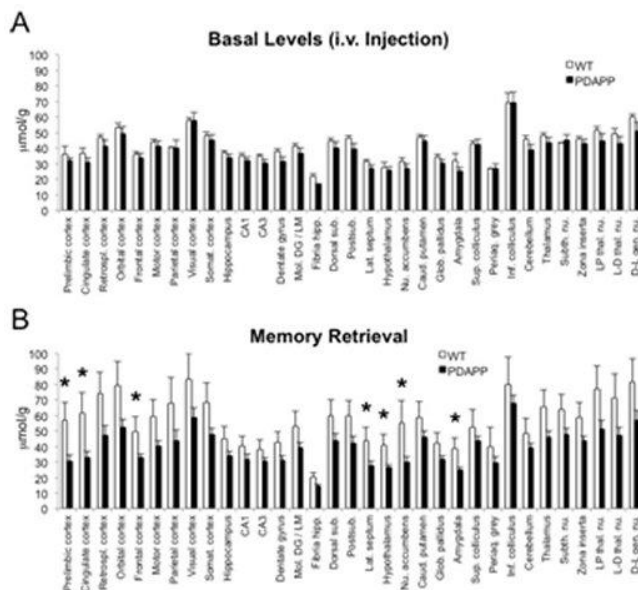
Mulder J, Zilberter M, Pasquaré SJ, Alpár A, Schulte G, Ferreira SG, Köfalvi A, Martín-Moreno AM, Keimpema E, Tanila H, Watanabe M, Mackie K, Hortobágyi T, de Ceballos ML, Harkany T. Molecular reorganization of endocannabinoid signalling in Alzheimer's disease. Brain. 2011 Apr;134(Pt 4):1041-60.

D1.3 Imaging studies to reveal brain areas of task-related activation.

Design: The design of the study involved (a) a series of watermaze phases coupled to the administration of [C-14-]-2-DG, and (b) subsequent blind quantification of the 2-DG signal in 33 brain structures by autoradiography. Different groups of animals went through one or more phases of the study, with the different phases reflecting baseline, memory encoding, learning to a specified criterion and finally memory retrieval, as described in the previous report.

Results: PDAPP mice showed a non-significant trend towards reaching criterion more slowly, but 24 hr and 7 days later, they displayed greater forgetting of spatial memory than controls (probe test). Analysis of the 2-DG images showed similar levels of significant but modest hypometabolism in separate PDAPP groups, relative to controls - at baseline, memory encoding, and consolidation (learning to criterion). However, strong differences were observed in the memory retrieval group, with PDAPP mice showing substantially lower glucose utilisation than WT mice in several brain structures (Fig. 1B), in stark contrast with the pattern in the baseline group (Fig. 1A).

Figure 1



Analysis of global glucose utilisation (across all brain structures analysed) revealed an increase in glucose metabolism in WT mice at memory retrieval, compared to the other memory phases, which was absent in PDAPP mice. These results suggest that the memory impairment in PDAPP mice at a pre-pathological age, which as we have shown previously is largely specific to the retrieval phase, is underlied by an equally retrieval-specific deficit in memory-associated glucose metabolism. Our analysis enables correlation of behavioural with physiological profiles, which could lead to a sensitive biomarker strategy for the early stages of Alzheimer's disease.

D1.4 Memory loss arising from exogenous Aβ administration

The objective of the research work was memory testing after exposure to Aβ peptides and drug-candidate treatment in rats and mice. Different synthetic Aβ aggregate species (oligomers and fibrils) were used in rats. A new drug candidate (BFR-106) was used as a neuroprotective agent against Aβ oligomers. The treatment of the efficiency of BFR-106 was tested (1) for memory effects in rats with acute Aβ-administration, and (2) for memory effects in transgenic mice (AD-model animals).

Summary of progress: Synthetic oligomeric and fibrillar Aβ 1-42 samples were prepared. Oligomeric Aβ was administered icv, fibrillar Aβ introduced intrahippocampally to wt rat brain. The effect of the two Aβ species on spatial memory was tested in Morris water maze. Both Aβ species (and both administrations) caused decrease in spatial memory. Administration both Aβ species resulted in a significant decrease in dendritic spine density compared to untreated controls.

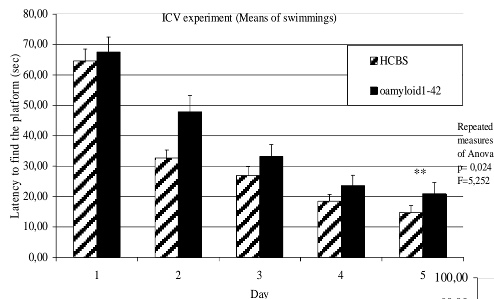


Fig.1: ICV injection of oAβ 1-42 resulted learning deficit. Performance is expressed as the mean (±S.E.M.) latency to find the platform during the 5 days period.

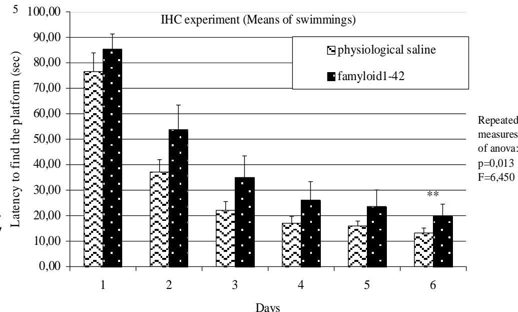


Fig.2: IHC injection of fAβ 1-42 resulted learning deficit. Performance is expressed as the mean (±S.E.M.) latency to find the platform during the 6 days period.

Novel peptidomimetics were designed and synthesized for protecting neuronal cells from the toxic effect of Aβ-species. After screening with MTT-assay compound BFR-106 was tested in vivo. BFR-106 showed neuroprotective effect against exogenous oligomeric Aβ in wt rats. Long (6 months) treatment of APPxPS1 tg-mice with the same compound prevented neurodegeneration (memory testing, neuronal viability, tau pathology, dendritic spine density).

1.5 Testing of novel drug treatments to counteract Aβ effects

See WP6.

WP2

Network Communication and Memory Loss

A. Objectives

The **specific objectives** of this Work Package are:

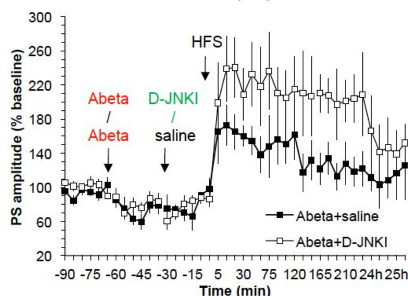
2.1. To address changes in synaptic plasticity and network activity at the field potential level in animals that have been treated with Aβ oligomers.

2.2. To investigate long-term effects on synaptic plasticity and network activity, as well as possible ameliorative effects of environmental enrichment and exercise.

2.3 To address whether peptides targeted against Aβ or portions of Aβ will ameliorate deficits in these phenomena

D2.8 Counteracting Abeta effects on LTP/LTD by anti-abeta drugs

We started work on this deliverable ahead of time, as we had the opportunity to test putative LTP-modulating peptides that were being developed by Michael Courtney (UKU). We began by assessing whether a peptide that inhibits c-Jun N-terminal kinase (JNK) alters synaptic plasticity and found that the inhibitor enhances long-term depression, inhibits depotentiation and does not affect LTP in the dentate gyrus of freely behaving rats. This finding was published in 2011. The observation that depotentiation is inhibited motivated us to test the peptide in our Abeta-treated animals. We observed that the peptide can indeed ameliorate acute LTP deficits if given at approximately the same time as Abeta treatment (see figure below). Now we are testing other peptides that act on the molecular elements of the LTP cascade.



The black symbols represent LTP that was evoked in animals that were treated with Abeta 60 min, and saline 30 min before tetanisation (HFS) was applied. Here, an initial synaptic potentiaion was produced that reverted to basal levels by 4h after the experiment had been commenced. The white symbols represent LTP that was evoked in animals treated with Abeta and the JNK inhibitor. Here a significantly larger LTP was elicited that persisted for well over 24h.

Yang H, Courtney MJ, Martinsson P, Manahan-Vaughan D. Hippocampal long-term depression is enhanced, depotentiation is inhibited and long-term potentiation is unaffected by the application of a selective c-Jun N-terminal kinase inhibitor to freely behaving rats. Eur J Neurosci. 2011 May;33(9):1647-55.

WP 3

A. Objective and tasks

The overall **objectives** of this work package are:

(1) to determine the ability of different conformations of Aß to rapidly disrupt synaptic plasticity mechanisms that are believed to underlie impairment of memory in Alzheimer's disease.

(2) to effectively target these species with novel potential therapeutic agents.

Whereas work package 2 focuses on the long term effects of Aß at the network level and delayed effects on synaptic plasticity in vivo, work package 3 explores the rapid acute disruptive effects of Aß on LTP/LTD both *in vivo* and *in vitro*.

Deliverable D3.1 Report on the acute effect of exogenous natural and synthetic Abeta on rat hippocampal LTP-LTD (TCD)

We have completed our comparison of the effects of acute application of synthetic Abeta species with different known conformations on hippocampal LTP with the most potent known form: natural oligomers from cell medium. Overall we found that Abeta oligomers derived from iso-Abeat1-42 or synthetic Abeta1-42 that contained fibrils (both supplied by USZ) had similar efficacy to natural Abeta dimers in terms of ability to disrupt hippocampal synaptic plasticity.

Thus intracerebroventricular injection of cell-derived and synthetic Abeta oligomers completely inhibited LTP of excitatory synaptic transmission at hippocampal CA3-to-CA1 synapses, measured 3 h after high frequency conditioning stimulation (Fig. 3.1). Using a four step purification protocol, consisting of anion-exchange followed by immuno-affinity, reverse phase and size exclusion chromatography, it was possible to isolate essentially pure Abeta monomer and dimer fractions in conditioned medium from cells (7PA2 cells) overexpressing amyloid precursor protein (APP) provided by the laboratory of Prof Dominic Walsh. Whereas the dimer fraction powerfully inhibited LTP the monomer fraction was inactive. This finding is similar to what we reported in our Year 1 Report for the effect of cell-derived oligomer and monomer enriched fractions that had been partially purified using a two-step protocol of ion-exchange and size exclusion chromatography. However, the potency of the synthetic Abeta1-42 oligomers was several fold lower than the potency of cell-derived Abeta oligomers. This difference in potency may arise because the synthetic preparation contains a wide range of oligomer sizes, from low-n to near-protofibril dimensions, whereas the cell-derived preparation contains primarily SDS-stable low-n oligomers of Abeta.

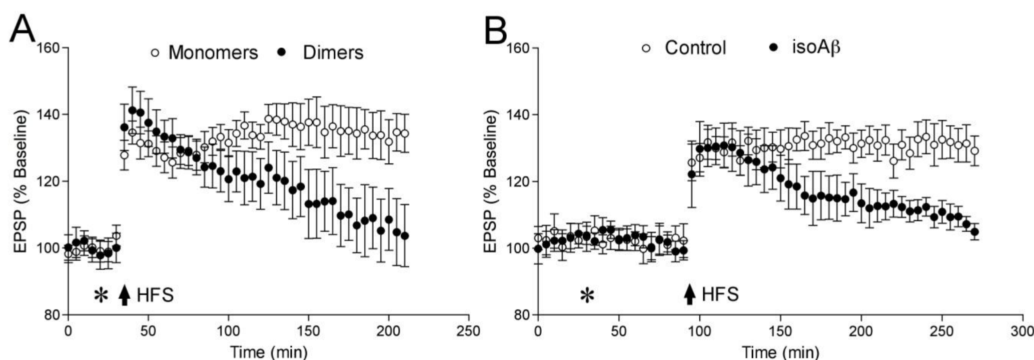


Fig. 3.1

Highly purified natural Abeta dimers and synthetic Abeta1-42 oligomers inhibit LTP in the CA1 area of the rat. (A)

Whereas application of the dimer-enriched fraction from the 7PA2 cell conditioned medium completely inhibited LTP ($p<0.05$), the momomer-enriched fraction was without effect ($p>0.05$). Similarly, synthetic Abeta1-42 prepared from iso-Aß completely inhibited LTP ($p<0.05$).

Somewhat similarly, injection of synthetic Abeta1-42 that contained fibrils fully inhibited LTP at 3 h (Fig. 3.2). In this case it was not possible to assess accurately relative potency because the fibril-containing preparations were very gelatinous thereby making comparison of actual quantities delivered more difficult than with the oligomer preparations. Moreover the inhibition of LTP by this preparation of Abeta was prevented by pre-incubation with an anti-oligomer antibody, A11. This finding indicates that oligomeric species contribute to the inhibition of LTP and are in dynamic equilibrium with the Abeta fibrils.

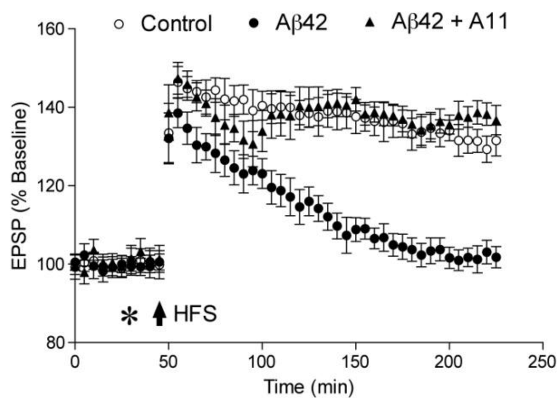


Fig. 3.2 Fibril-containing synthetic Abeta1-42 inhibited LTP. Non-washed "fibrillar" pre-aggregated Abeta42 inhibition of LTP ($p<0.05$) was prevented by the anti-oligomer antibody A11.

Deliverable D3.2 Report on the acute effect of exogenous Aß on hippocampal LTP-LTD in ApdE9 versus wild type mice (TCD, UEF)

In order to test the hypothesis that Abeta fibrils can neutralize and reduce the inhibition of LTP by Abeta oligomers we (TCD) have carried out studies on the relationship on the synaptic plasticity effects of washed Abeta fibrils in detail. We found that repeated washing of Abeta1-42 fibril-containing preparations resulted in a loss of ability to inhibit LTP (Fig. 3.3), consistent with the view that fibrils in the absence of more mobile oligomers are insufficient to disrupt synaptic plasticity under our experimental conditions. Furthermore washed Abeta fibril-containing preparations from brain plaques behaved in a similar manner (Fig. 3.3).

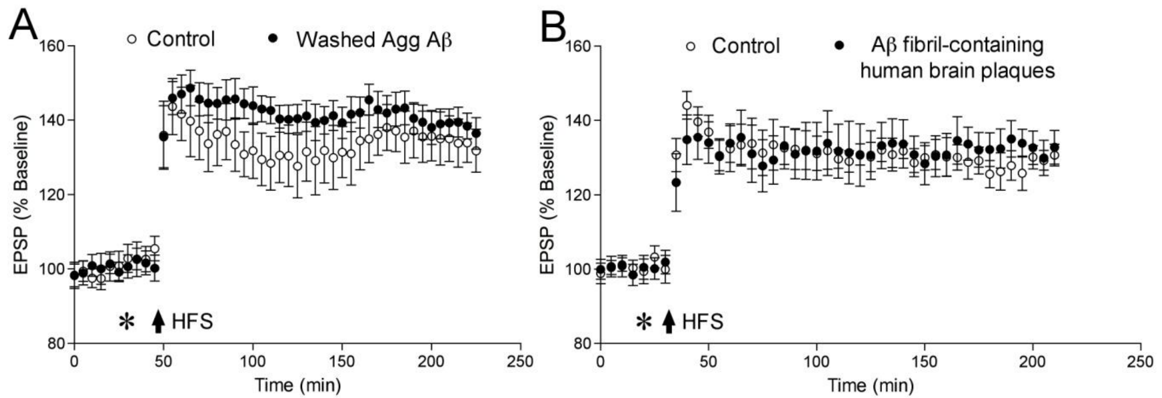


Fig. 3.3 Washed Abeta fibrils fail to inhibit LTP. (A) Pre-aggregated Abeta1-42 fibrils that had been subjected to repeated washing did not inhibit LTP ($p>0.05$). (B) Similarly Abeta fibril-containing brain plaque that had been repeatedly washed had no significant effect on LTP ($p>0.05$).

Objectives: (1) to determine the ability of different conformations of Aß to rapidly disrupt synaptic plasticity mechanisms that are believed to underlie impairment of memory in Alzheimer's disease.
(2) to effectively target these species with novel potential therapeutic agents. During the reporting period TCD was primarily responsible for WP 3 with the help of partners in UEF and USZ. Overall every aspect is reasonably well on track with some unavoidable delays.

During the reporting period WP3 focused on meeting the relevant milestone and deliverables related to the discovery of new means of targeting the synaptic plasticity disrupting Aß using peptidomimetics and the relative role of plaques in modulating the disruptive effects of Aß oligomers. The main findings are illustrated in the figures. Thus, an Aß1-42 – mediated inhibition of LTP (Fig. 1A) was prevented by co-administration of an anti Aß oligomer peptidomimetic (Fig. 1B). Somewhat similarly, an anti-Aß fibril peptidomimetic abrogated the inhibition of LTP by Aß1-42 (Fig. 2B) but in this case the compound alone was able to inhibit LTP (Fig. 2A).

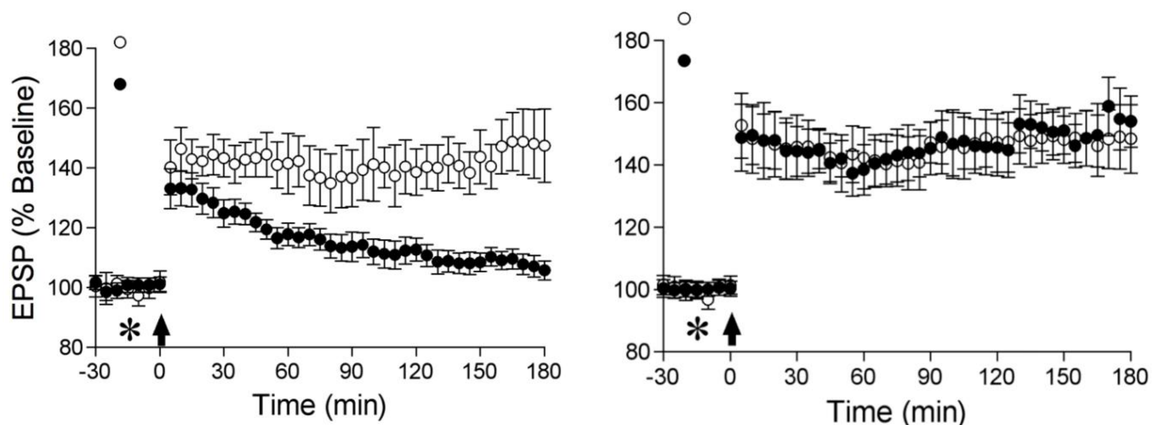


Fig. 1 (left) Aß1-42 prepared from isoAß powerfully inhibits LTP induced by high frequency stimulation (arrow) in the CA1 area of the hippocampus of the urethane anaesthetized rat. Animals were injected

(asterisk, 5 µL i.c.v.) with either vehicle (open circles, n=6) or Aß1-42 (closed circles, 900 pmol, n=7). (rigth) The anti-oligomer peptidomimetic P29 completely abrogated the inhibition of LTP by Aß1-42. Animals were injected (asterisk, 5 µL i.c.v.) with either P29 alone (open circles, 90 nmol, n=4) or P29 + Aß1-42 (closed circles, 900 pmol, n=5).

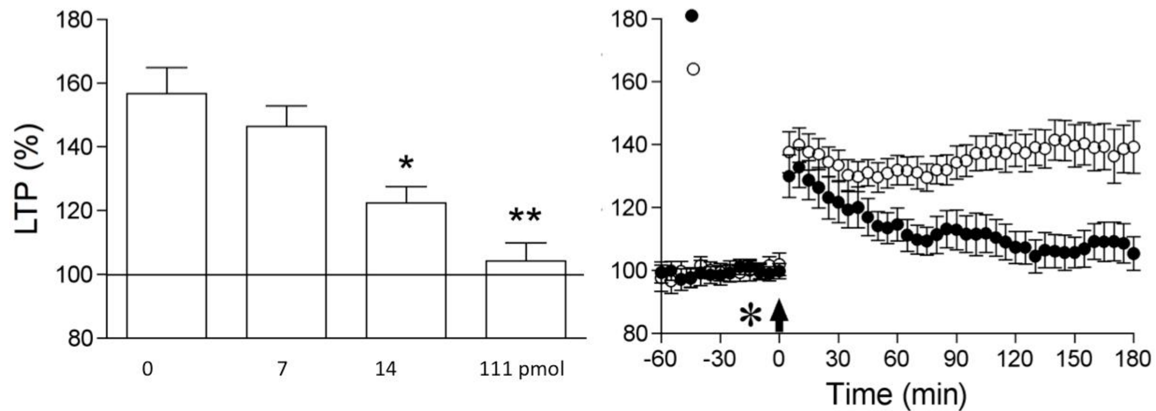


Fig. 2 (left) The test anti-Aß fibril peptidomimetic caused a dose-dependent inhibition of LTP. The bars represent the magnitude of LTP measured 3 h after high frequency stimulation in animals injected i.c.v. with either vehicle (n=7), 7 nmol (n=4), 14 nmol (n=3) or 111 nmol (n=4) of the test peptidomimetic. *P<0.05, **P<0.01. (right) The test anti-Aß fibril peptidomimetic, at a dose which on its own did not inhibit LTP, completely prevented the inhibition of LTP by Aß1-42. Animals were injected i.c.v. with either Aß1-42 alone (closed circles, 500 pmol, n=7), or Aß1-42 + 7 nmol test compound (open circles, n=8)

WP4

Objectives: the study of molecular mechanisms leading to the elimination of cortical (and hippocampal) synapses in the cascade of neurodegenerative events clinically manifesting as Alzheimer's disease. The cerebral cortex is emphasized here since Alzheimer's disease is causally linked to impaired synaptic signalling in neo- and archicortical areas. APdE9 mice or acute beta-amyloid (Aβ) superfusion are used to model Alzheimer's-like pathological states, imposing synaptic deficit. WP4 contains logically-linked milestones performed by KIS, in association with UKU. This (third) reporting period – from August 2010 until January 31, 2012 – focuses on deliverables 4.1 (KIS), 4.2 (UEF), 4.3 (UEF) and 4.5 (KIS). Data on modifications of cortical endocannabinoid-mediated DSI/DSE in the presence of Aβ have been published (4.5, see: Mulder *et al.*, Brain (2011) 134:1041-1060). This periodic report also describes initial results in relation to deliverable 4.6 (KIS) on the "role of neuronal energy metabolism in maintaining neuronal excitability in Alzheimer's disease". Deliverables 4,1 and 4.5 are completed (and published Minkeviciene *et al.*, 2009; Mulder *et al.*, 2011). Deliverables 4.2 and 4.3 are in progress at 30% and 50% of completion, respectively. Deliverable 4.6 is in progress, too. Although it is estimated as "100% complete", follow-up as per reviewer comments during peer-reviewing of the relevant dataset is underway.

Progress per deliverable:

D.4.1 (months 1-48 [KIS], *completed*). The analysis of excitatory synapses has been completed and reported earlier ("30 month report"). These data are the basis of our studies in D4.8, aimed to rescue errant excitatory network activity in APdE9 mice and under acute Aβ exposure. We have approached Aβ effects on GABAergic synapses in two ways: 1) Ca^{2+}-binding proteins (CBPs) are selective markers of subpopulations of GABAergic interneurons in the cerebral cortex. CBP expression has been thought to protect neurons against Aβ toxicity (parvalbumin and calretinin expression is relatively spared in Alzheimer's brains; Hof *et al.* 1993/1994), particularly when an excitotoxic cascade of Aβ toxicity is implicated in neuron damage (Mattson *et al.* (1992) J Neurosci.). We have recently identified a fourth neuron-specific CBP in the mammalian brain (secretagogin; Mulder et al. (2009) PNAS). This finding allowed us to use secretagogin as a molecular "probe" of previously unidentified GABA cell subpopulations in Alzheimer's brains and APdE9 mice. We find that secretagogin-expressing neurons are resistant to Aβ accumulation in APdE9 mice. By looking at adult-born neurons and migrating towards the olfactory bulb we find histochemical indices of unperturbed migration even in areas with high Aβ load (Fig. 1). Differentiated neurons in Alzheimer's brains (particularly in the olfactory system and hippocampus) were found resistant to Alzheimer's pathology. However, we have identified an unconventional "human-specific" secretagogin$^+$ cell population in the human olfactory tract, which succumbs to Alzheimer's pathology (cytoskeletal modifications/tau accumulation), identifying a novel cellular locus of olfactory dysfunction in Alzheimer's disease (Attems, Alpar *et al.* (2012) PNAS). 2) Studies at the level of synapse neurophysiology showed that perisomatic GABAergic synapses are sensitive to acute Aβ. However, and in contrast to excitatory neurotransmission, we find that Aβ prolongs endocannabinoid signalling at GABAergic terminals by increasing the recruitment of CB_1 cannabinoid receptors at the cell surface. This leads to prolonged depolarization-induced suppression of inhibition (DSI) (reported in: Mulder *et al.* (2011) Brain; Fig. 4). This effect can decrease the probability of GABA release, effectively decreasing inhibition in hippocampal neuronal networks. The subsequent loss of excitation/inhibition balance can manifest as epilepsy in APdE9 mice

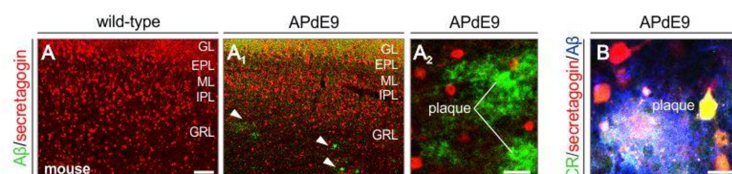


Fig. 1 – **Secretagogin-expressing neurons in the APdE9 mouse brain**. (A,A_1) Overview of the olfactory bulb (periglomerular region) of adult wild-type and APdE9 mice. Note the lack of secretagogin$^+$ cell loss or lamination defect in APdE9 samples. (A_2) Secretagogin$^+$ neurons reside in the close proximity of senile plaques, and (B) retain neurochemical diversity as in wild-type littermates. *Abbreviations*: CR, calretinin; GL, glomerular layer, GRL, granular layer; IPL, internal plexiform layer; ML, mitral layer. Modified from Attems, Alpar *et al.* (2012).

(and Alzheimer's patients). Cumulatively, these studies show that Aβ stimuli disrupt synaptic plasticity of cortical GABAergic interneurons.

D4.2 (months 48 [UKU], *completion: 30%*). This deliverable is aimed to establish an assay to study the impact of Aβ on the trafficking of glutamate receptor (NMDA; AMPA) subunits in cultured cortical neurons.

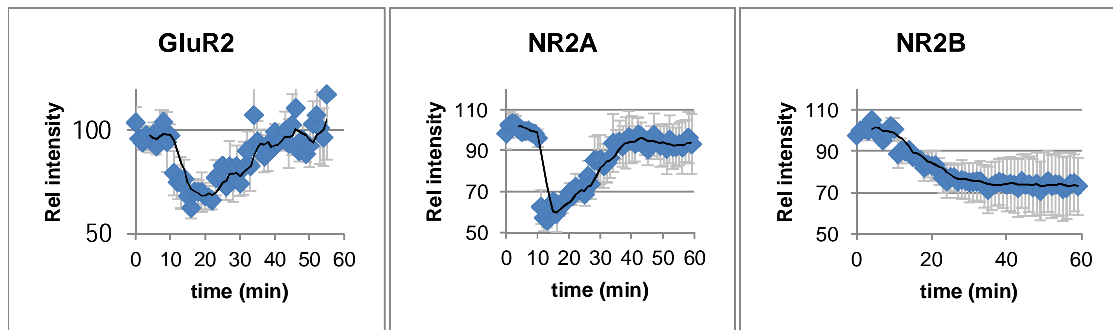


Fig. 2 – **GluR2, NR2A and NR2B receptor internalization.** NR2B subunit does not recover to the membrane. The black lines are trendlines ($n = 3$).

In vitro mature primary cultures (>12 days *in vitro*) are transfected by superecliptic phluorin, a pH-sensitive GFP construct fused with glutamate receptor subunits (e.g., GluR2, NR2A and NR2B). Total Internal Reflection Microscopy (TIRFM) is then used to detect receptor subunits specifically at the plasma membrane, and to follow the internalization/recycling of glutamate receptors triggered by NMDA and sequentially followed by drug wash-out (or alternatively inhibiting the NMDAR by MK801). All investigated receptor subunits respond to 50µM NMDA/10µM glycine. GluR2 or NR2A but not the NR2B subunit recovers to the membrane (Fig. 2). This allows molecular studies directly related to "MEMOLOAD" to next assay the impact of Aβ (oligomers and fibrils) on the rate of the recovery of receptor chimeras. Our overarching question - if there is an Aβ effect – is whether we can challenge the Aβ-induced modifications to receptor recovery by selective inhibitors that disturb interactions of the receptor subunits with downstream effectors. One particular inhibitor to be tested is the peptide NR2B9C, which inhibits receptor/MAGUK interaction; because we find indications on Aβ influencing MAGUK levels (*see below)*. Expected date of completion: 30/11/2012.

D4.3 (months 24-48 [UKU], *completion: 50%*): This deliverable addresses the stability of the post-synaptic density (PSD) before and after treatment with Aβ oligomers. Aβ oligomers were prepared by 24h pre-incubation, and supefused on cultured cortical neurons. PSD95 protein, one of the major scaffolding proteins in the PSD that interacts with glutamate receptors and downstream effector molecules, was chosen as target. To study PSD95 stability, we fused the reversible photoactivatable protein Dronpa with the PSD95 C-terminus, and used this construct to detect PSD95-Dronpa protein by photoactivation in a dendritic spine region (Fig. 3A). The advantage of using Dronpa (relative to GFP or photoactivatable GFP, reported previously) is that Dronpa can be repeatedly photoactivated and erased, increasing interpretability and improving data quality. The decay of photoactivated PSD95 protein before the addition of Aβ oligomers was compared with the decay of photoactivated PSD95 protein after 15 min incubation

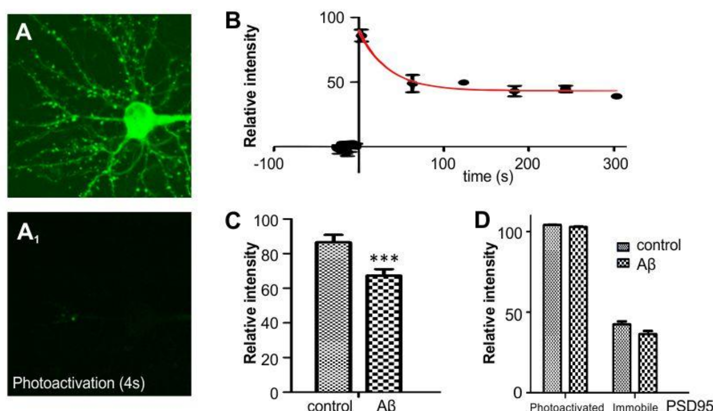


Fig. 3 – **Aβ effect on PSD recruitment and mobility** (A) Clusters of PSD95 in a cortical neuron used for photoactivation. (A_1) First image (4s) after photoactivation of a dendritic spine region of the same neuron as in (A). (B) Fitting (one-exponential decay) of data from PSD95 photo-activation experiment ($n = 3$). (C) Comparison of the level of photo-activated PSD95 protein in a dendritic spine region 15 min after addition of 3µM Aβ (24h pre-incubation) compared with control (15 min after addition of Neurobasal medium) in cortical neurons (13-15DIV). The levels are normalized to the first data point after photoactivation before the edition of Aβ or neurobasal medium. $***p < 0.001$. (D) Comparison of the immobile fraction of PSD95 molecules in a dendritic spine region 15 min after 3µM Aβ incubation with control (incubation with vehicle) in cortical neurons.

(Fig. 3B). Two parameters (of the one exponential decay of PSD95) were significantly different upon Aβ exposure when compared to control samples (exposed to neurobasal cell culture medium): 1) the *initial amount of PSD95 protein* that could be photo-activated in a selected dendritic spine region (corresponding to the relative PSD95 content in dendritic spines); 2) the *immobile fraction of the PSD95 protein* that was initially photo-activated (fraction of spine PSD95 that is stably tethered to the spine) (Fig. 3C,D). The t½ of the decay, representing the mobile fraction of spine PSD95 did not change significantly in response to Aβ treatment (Table 1). Thus, in on-going experiments, a novel assay system is used to address the relationship of Aβ conformers to PSD expression and stability in dendritic spines. Expected date of completion: 30/11/2012.

Table 1. Summary of parameters shown In Fig. 3C,D

	amount of photoactivated PSD95 proteins 15min after adding 3μM Aβ/NB medium compared with before addition	immobile fraction (normalized to number of photo-activated PSD95 proteins
Aβ (24h oligomeric)	67.3	36.2
NB	86.6	42.3
p-value	<0.001 ***	<0.01 **

D4.5 (months 36 [KIS], *completed*): Endocannabinoid (2-AG)-mediated synaptic signalling is critical to control the efficacy and plasticity of neurotransmission at many cortical and hippocampal synapses. We have performed immunohistochemistry (Mulder *et al*., 2011) and autoradiography (Farkas *et al*., 2012) studies to show largely unchanged CB_1 cannabinoid receptor levels and binding activity in *post-mortem* Alzheimer's brains. By establishing the coincidence of increased 2-AG generation due to increased and ectopic *sn*-1-dyacylglycerol lipase (DAGL) expression (that is, DAGLs can be found in activated microglia in Alzheimer's brains) and impaired recruitment of monoacylglycerol lipase (MAGL, the major 2-AG degrading enzyme) we have provided molecular evidence suggesting increased 2-AG availability in Alzheimer's brains. Our notion is supported by a recent report establishing positive correlation between lowered brain anandamide concentration (another endo-cannabinoid) and Kendrick's digital copy or Boston naming tests (Jung *et al*. Neurobiol Aging (2011) May 3 (ahead of print)). We performed DSI measurements in the hippocampus upon Aβ superfusion and find significantly prolonged synaptic suppression, as compared to controls (Fig. 4). In collaboration with Andrew Randall (University of Britsol, UK), we have followed-up our finding with acute Aβ application in APP/PS1 tranegnic mice. However, it appears that the relative contribution of endo-cannabinoids to controlling synaptic plasticity decreases during adulthood in mice (synaptic responses became variable and DSI/DSE

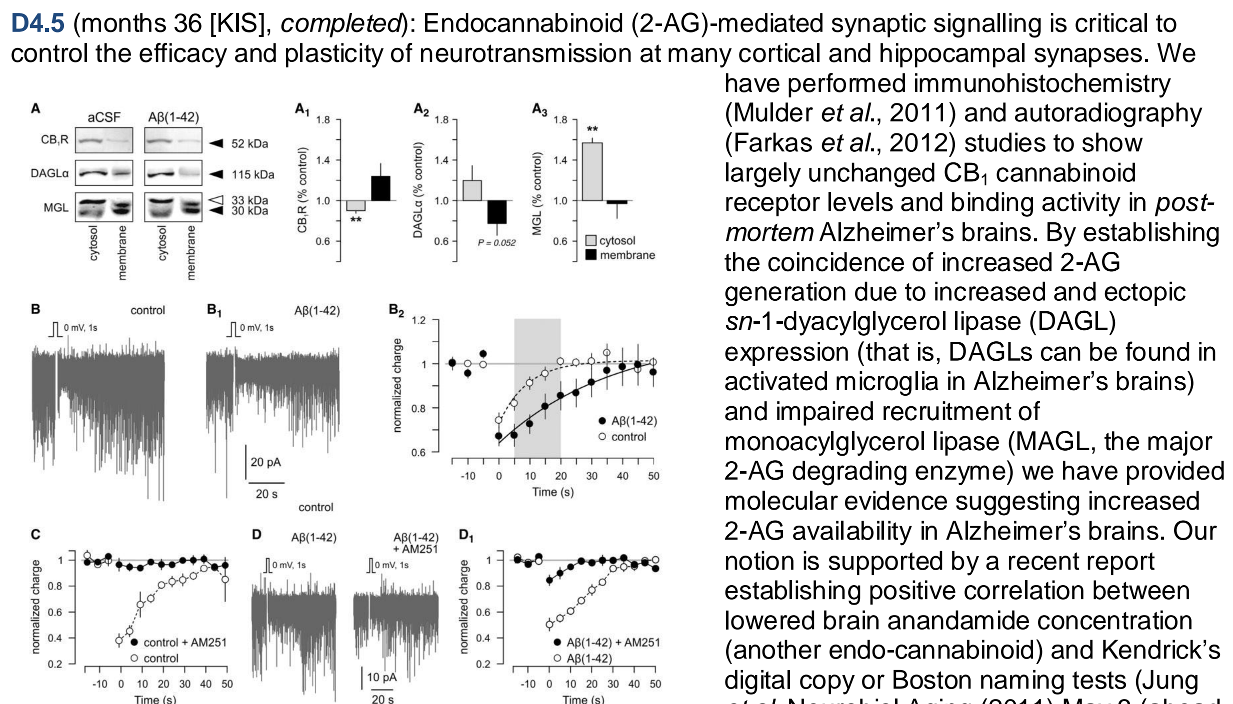


Fig. 4 - **Aβ1–42 triggers subcellular redistribution of 2-arachidonoyl glycerol signalling networks and prolongs depolarization-induced suppression of inhibition**. (A–A_3) Representative images of molecular targets resolved by Western blotting. Cytosol and membrane fractions are from the same sample to establish the impact of Aβ1–42 on subcellular receptor and enzyme recruitment, as compared to controls in artificial cerebrospinal fluid (aCSF). Aβ(1-42) exposure significantly alters intracellular *vs.* cell surface concentrations of CB_1 cannabinoid receptors (A_1), DAGLα (A_2) and monoacylglycerol lipase (A_3). Data on monoacylglycerol lipase (MGL) show cumulative changes in this enzyme's 33 and 30 kDa isoforms. (B) Representative experiment showing depolarization-induced suppression of inhibition induced by a step depolarization of an Ammon's horn 1 pyramidal neuron. (B_1) Postsynaptic depolarization-induced reduction of spontaneous inhibitory postsynaptic currents (downward deflection from baseline) is prolonged by bath-applied Aβ1–42 (1 μM). (B_2) Summary of depolarization-induced suppression of inhibition magnitude and kinetics measured in control (open circles; n = 10 cells) or in the presence of Aβ1–42 (solid circles; n = 11). AM251 (1 μM) abolishes depolarization-induced suppression of inhibition in control (C), as well as Aβ1-42-superfused Ammon's horn 1 pyramidal neurons (D and D_1). **p < 0.01 (Student's t-test). Data were expressed as means ± SEM.

was difficult (if at all possible) to evoke). Therefore, we have discontinued the large-scale analysis of APdE9 mouse cohorts. Nevertheless, our above finding on increased DAGL activity may be pharmacologically significant since DAGL may generate neuroactive lipids other than 2-AG. We plan to address this possibility by combining pharmacological inhibition of DAGL activity and mass-spectrometry determination of lipid species ("target discovery").

D4.6 (months 48-59 [KIS], *completion: approx. 100%*): This is a new deliverable that had replaced an earlier aim since the behavioural phenotypes of the compound mutant mice we have initially proposed to study (APdE9::TrkB.T1 (loss-of-function) or APdE9::TrkB (gain-of-function)) did not justify neurophysiology studies on the interplay of brain-derived neurotrophic factor/TrkB signalling and Aβ in relation to synapse integrity and signalling. A series of recent findings in our and our collaborator's laboratories suggest that neurons can efficiently utilize oxidative energy substrates other than glucose (see the attached list of publications for details). In particular, lactate, pyruvate and ketone bodies (e.g., 3-β-hydroxybutyrate; BHB) can sustain neuronal energy demands upon neuronal network activity. Therefore, our overarching goal is to demonstrate whether enhanced neuronal energy (ATP) production and utilization by dietary complementation of glucose can protect and/or rescue neurons from Alzheimer's-like pathology and synaptic deficits. Our experiments include the acute application of Aβ conformers, as well as APdE9 transgenic mice. *Acute electrophysiology experiments were completed as per the relevant phasing of milestones* (Fig. 5). In addition, we have achieved significant advances in the behavioural analysis of APdE9 mice fed with pyruvate/BHB-supplemented diet). We found neuronal hyperexcitability in the *ex vivo* hippocampi of APdE9 mice underscored by significantly depolarized resting membrane and GABA-mediated current reversal potentials (Fig. 5D,E). Altered membrane properties and ionic conductances were specific to neuronal sub-populations. APdE9 mice exhibit

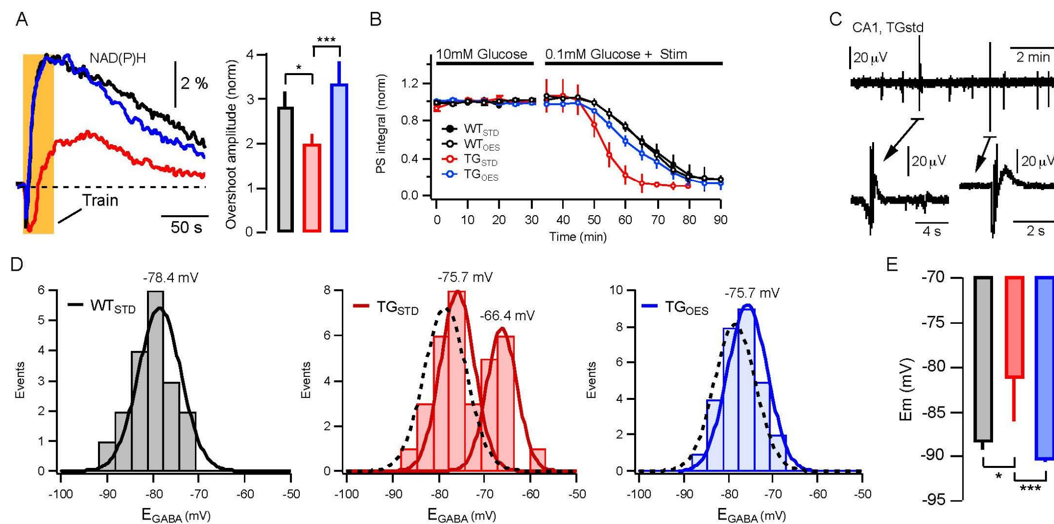


Fig. 5 – **Modification of electrophysiological and metabolic parameters in APdE9 mice is prevented by an energy-rich (OES) diet.** (A) Examples of original NAD(P)H signalling induced by 30s stimulation in wild-type slices (black), APdE9 on standard diet (red) or APdE9 on energy-rich diet (blue). The graph shows summary of overshoot (signal above baseline) amplitudes normalized in each experiment to the amplitude of oxidative phase (signal below baseline). (B) Feeding of oxidative energy sources improves neuronal tolerance to hypoglycaemia as seen by the population spike decay kinetics after reduction of the glucose concentration from 10 mM to 0.1 mM in artificial cerebrospinal fluid. (C) Aβ1-42 induces paroxysmal network activity in hippocampal CA1 region in glucose-only ACSF. (D) Distribution of E_{GABA} in dentate granule cells in slices from wild-type and APdE9 mice (TG) fed by either standard (STD) or enriched (OES) diets. Wild-types revealed similar results on both diets. Distributions were fitted with Gaussian. For comparison, the dotted curves depict the wild-type distribution. Note that OES diet normalized the E_{GABA} distribution in APdE9 mice. (E) The neuronal resting potential is significantly depolarized in APdE9 mice on standard diet. OES diet (blue) rescues this effect. $*p < 0.05$, $***p < 0.001$.

spontaneous epileptic seizures and other electroencephalographic epileptiform discharges. Dietary supplementation of oxidative energy substrates attenuated the probability of epileptiform activity in transgenic mice. We have linked altered neuronal energy metabolism upon Aβ application to discordant population activity by simultaneously recording transients of oxygen partial pressure and NAD(P)H utilization (see also Fig. 5A) during intense neuronal activity. Aβ induced the imbalance of energy

metabolism by increasing the oxidation area in the NADH signal. Oxidative energy substrates prevented Aβ-induced changes to metabolic parameters. These data extend our earlier findings (Minkeviciene *et al*., 2009) by showing that Aβ-induced neuronal hyperexcitability is due to disrupted energy metabolism, and suggest that Alzheimer's-related neuronal abnormalities can be reversed by dietary supplementation with oxidative energy substrates. These studies will be complemented by determining the effects of dietary supplementation with pyruvate/BHB on 1) brain glucose/glycogen stores/biochemistry, 2) protein networks in the Krebs-Szentgyörgyi and tryglyceride cycle/proteomics, 3) adaptive changes in neuronal structure (synapse densities) and excitability (maintenance of excitability under intense network activity).

Cumulatively, these studies (particularly future Aims #2 and #3) will allow us to propose an *alternative to the peptidomimetic rescue strategy originally proposed as deliverable 4.8*. Our alternative approach will be particularly appealing if the design and/or validation of an *in vivo* efficacious anti-Aβ peptidomimetic were to fail. Completion by: 31/1/2013.

Published data:

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WP 5

A. Objective and tasks

The **objective** of WP5 is to make a comprehensive assessment of the role of the MAPK/ERK and PI3K/Akt signalling pathways in the development of the pathology of AD and their contribution to the dysfunction of memory processing associated with the disease.

One of the principle aims of WP5 was to determine whether the Alzheimer's pathology is associated with dysfunctional regulation of the MAPK/ERK and PI3K-Akt signalling pathways. This is based on the important role that the MAPK/ERK signalling pathway in activating genomic responses necessary for long term plasticity associated with memory encoding and processing and that of the PI3K-Akt signalling pathway in homeostatic regulation of functions such as protein and glycogen synthesis, cell survival and growth, transcription, ect, necessary for neuronal viability. To this end we used two different murine models of AD, a PS1/APP and the PDAPP transgenic mouse lines, a rat model to investigate the role of toxic, soluble, oligomeric species of beta amyloid in comparison with post mortem tissue from AD patients.

The main finding was that oligomeric amyloid, infused icv over 7 days in the rat brain showed a decrease in phosphorylation of Akt 3 days following termination of treatment and a decrease in Akt protein levels by 4 weeks. It also induced a temporary deficit in spatial recognition memory, lasting approximately 40 days after the end of treatment, thereafter the deficit slowly recovered over period of 111 days post treatment to the same level as that of the control rats. In contrast rats infused with the amyloid peptide and an inhibitor of activity of Akt, LY294002, at a concentration that, alone had no effect, induced a more enduring deficit that lasted on the order of at least 4 months (See Figure 1).

The deficits induced by the combined effect of infusing beta amyloid for 7 days and inhibiting Akt signalling for the first 3 of these days could be rescued by environmental or pharamacologically activating the PI3K-Akt signalling pathway. IGFII acts via insulin receptors that inturn activate Akt and has been shown to improve memory in normal rats (see Chen et al., ????). A single injection of IGFII at a sufficiently low dose to not effect control rats reversed the deficit such that rats performed as well as control rats. This amelioration of the deficit was long lasting, for at least 4 months following IGFII treatment (See Figure 2).

Environmental enrichment is known to have beneficial effects on memory and synaptic plasticity. Following an enrichment protocol in which we have previously shown rats have better memory that home cage control rats, we found that rats treated with beta amyloid and LY294002 displayed better memory performance compared with home cage

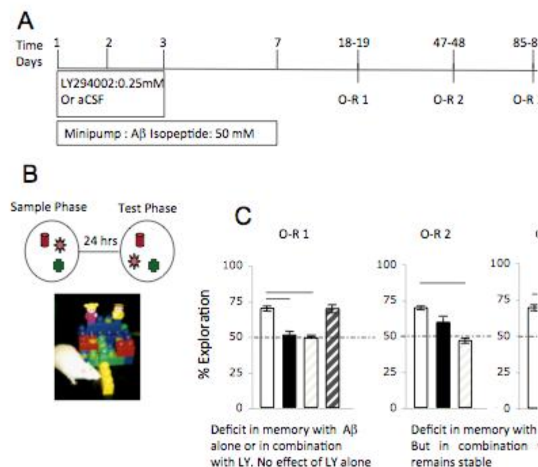

Figure 1: Time course of effect of Aβ isopeptide and LY294002 on spatial re
(A) depicts the time frame of experimental protocol, (B) depicts the rec
and (C) Shows impairment in memory perfomance in spatial recognition r
with isopeptide alone has a temporal restriction where the deficit is amel
months following treatment. In contrast when isopeptide treatment is c
of LY294002, which alone has no effect on memory perfomance, prolon
for at least 4 months.

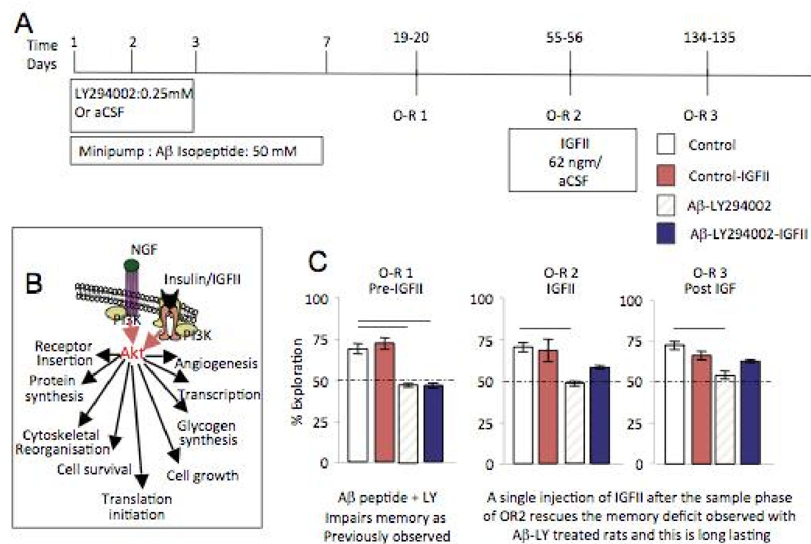

Figure 2: ICV injections of IGFII rescues memory deficits induced by Abeta isopeptide and inhibition of Akt signalling.
(A) depicts the time frame of experimental protocol, (B) shows potential functional regulation of IGFII activation of Akt signalling. (C) Shows impairment in memory perfomance in spatial recognition memory in rats treated with Abeta and LY294002 and subsequent improvement of deficits following IGF treatment.

controls and that which was equal to control rats. This improvement in memory, in contrast that see with IGFII, was short lasting, approximately 2 weeks following the end of enrichment, (See Figure 3).

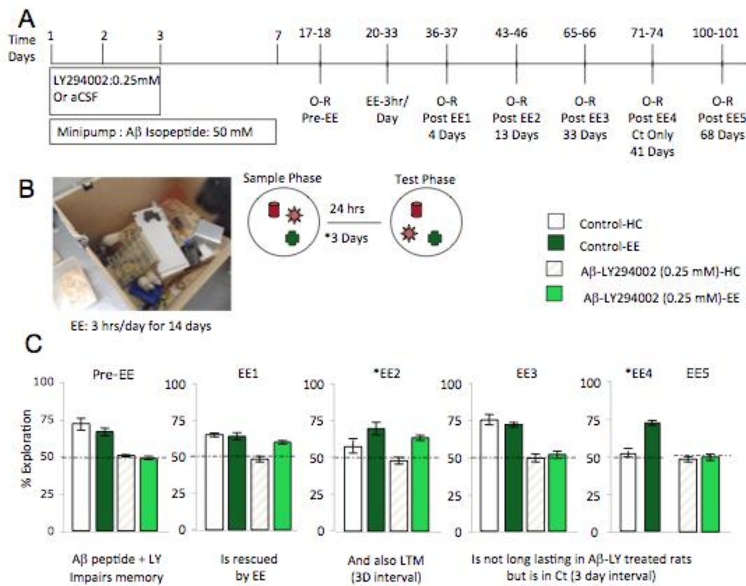

Figure 3: Environmental enrichment rescues memory deficits induced by Abeta isopeptide and inhibition of Akt signalling, but only on a temporary basis.
(A) depicts the time frame of experimental protocol, (B) shows enrichment and behavioural protocol. (C) Shows impairment in memory perfomance in spatial recognition memory in rats treated with Aβ-LY294002 prior to enrichment and subsequent improvement of memory deficits even with a 3 day delay between sample and test phases, following enrichment. This beneficial effect, however is temporary in Aβ-LY294002 as memory performance is at chance level several months after enrichment, compared with control rats which show continued improved performance with a three day interval compared with home-cage controls.

The importance of these finding suggest that (a) toxic oligomeric species of amyloid do impose a detrimental behavioural phenotype although not enduring. (b) This can be exacerbated by mildly inhibiting Akt signalling, and (c) by activating the signalling pathway; the memory deficit can be reversed. This lends strong support to the accumulating evidence that suggests circulating toxic amyloid bestows a more detrimental effect on memory than the aggregated species and that it interacts with Akt signalling function.