UNIVERSITY OF KUOPIO
Department of Neuroscience and Neurology

THE FIRST KUOPIO ALZHEIMER SYMPOSIUM UNDER THE AUSPICES OF DEPARTMENT OF NEUROSCIENCE AND NEUROLOGY, UNIVERSITY OF KUOPIO, KUOPIO, FINLAND

Proceedings of the Symposium

28-30 January 1999

Kuopio, Finland

THURSDAY, JANUARY 28

Session 1: What is important in the pathogenesis of Alzheimer's disease

Understanding the physiological function of APP - Implication for therapy of Alzheimer's disease
-Konrad Beyreuther

No neuronal degeneration, no Alzheimer disease
-Khalid Iqbal

The effects of apoE genotype on neuronal maintenance and repair: implications for therapy of Alzheimer's disease
-Danny Michaelson

Session 2: The role of inflammation in Alzheimer's disease

Is Alzheimer amyloid part of an inflammatory cascade?
-Huntington Potter

Inflammatory mechanisms and apoptosis in Alzheimer's disease
-David Cribbs

Session 3: The role of estrogen in Alzheimer's disease

Estrogen receptors and signalling cascades
- Pirkko Härkönen

Estrogen induced genes in rat hippocampus
-Elisabetta Vegeto

Effect of estrogen on neuronal plasticity
- Thomas van Groen

Estrogen and cognition
- Paavo Riekkinen Jr

FRIDAY, JANUARY 29

Special Lecture
Cholinesterase inhibitors in Alzheimer's disease: mechanism of action and preclinical profile
-Albert Enz

Session 4: Inflammation and mechanisms of neurodegenration in Alzheimer's disease

Glial cells in aging and dementia
- Irina Alafuzoff

The role of complement in Alzheimer's disease
-Piet Eikelenboom

Glial cell response in transgenic animal models
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The relationship between neurodegeneration and cell-cycle-related events in Alzheimer's disease
-Thomas Arendt

The role of COX-2 in Alzheimer's disease brain
- Lap Ho

Regulation of COX-2 expression in brain injury
- Jari Koistinaho

Cyclooxygenase in Alzheimer's disease: roles in brain inflammation and neurodegeneration
-Kerry O'Banion

Postmortem neurochemical changes associated with non-cognitive behaviour in Alzheimer's disease
-Paul Francis

AD-related proteins and synaptic markers in rats with entorhinal cortex lesions
- Maria-Javier Ramirez

HOECHST MARION ROUSSEL SYMPOSIUM; The role of glial cells in inflammation, pathogenesis and therapy of Alzheimer's disease

Brain inflammation and aging
- Beatrice Hauss-Wegrzyniak

Activated microglia in dementia
- Irina Alafuzoff

Prevention of glial response with propentofylline - In vitro and in vivo models
- Amanda McRae

Long term studies with propentofylline in Alzheimer type and vascular type of dementia
- Barbara Kittner

Pharmacoeconomical impact of long term propentofylline treatment in Alzheimer's disease
- Anders Wimo

SATURDAY, JANUARY 30

Session 5: Molecular pathogenesis of Alzheimer's disease

The genetics of dementia, from presenilins and amyloid to tau
- John Hardy

Transgenic animal models as tools to test current and new therapies for Alzheimer's disease
- Karen Duff

Studies on the cortical features of mice and rat transgenic Alzheimer's disease models
- Claudio Cuello

Variant Alzheimer's disease with "cotton wool" plaques
- Matti Haltia

Cholesterol metabolism, APP transport and processing
- Konrad Beyreuther

Presenilins and apoptosis - a key event in the pathogenesis cascade of Alzheimer's disease?
-Laurent Pradier

Tauopathies and dementia
-Inge Grundke-Iqbal

Session 6: Therapeutic strategies for Alzheimer's Disease

Anti-inflammatory agents in Alzheimer's disease
- John Breitner

Management of cognition and function: results from the clinical trials program of donepezil
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Metrifonate as a new treatment for Alzheimer's disease - Clinical profile
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Cholinesterase inhibitors are Alzheimer disease stabilizers
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Symptomatic or preventive therapy for Alzheimer's disease - Long term experience
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The Poster Session

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Special Lecture

Cholinesterase Inhibitors In Alzheimer's Disease: Mechanism Of Action And Preclinical Profile

Albert Enz

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Today the only approved therapy for Alzheimer's disease (AD) is the symptomatic treatment with acetylcholinesterase inhibitors (AchE-I). Since the first generation of AchE-l's, newer drugs became available and are in clinical use today. These second generation inhibitors bear advantages, mainly evidenced in a reduced potential for side effects.

Beneficial versus adverse effects associated with AchE-l's are determined by their inhibitory mechanism, pharmacokinetic properties and preferential site of action:
- The mechanism of inhibition mainly determines the duration of the interaction with the enzyme
- The potential for organ toxicity is due to the generation and presence of active metabolites and is determined by the metabolism of the drug
- Peripheral cholinergic side effects can be reduced by the use of brain-selective inhibitors.

Rivastigmine (Exelon®), an cholinesterase inhibitor of the second generation, is effective in preventing the enzymolysis of ACh. Rivastigmine has a long duration of action, central selectivity and few peripheral side-effects. No drug-drug interactions related to metabolism are known, because it is metabolized exclusively by the target enzymes (cholinesterases). In animals rivastigmine inhibits AChE in the

brain much more potently than in peripheral organs and consequently has minimal peripheral side effects. In addition the drug is more potent in inhibiting AChE in cortex and hippo campus than in other brain regions, such as striatum and pons/medulla. Assuming that these results can be extrapolated to humans, the observed selectivity would clearly be an advantage, since hippocampus and cortex are considered to be the main target regions for symptomatic treatment of AD.

The existence of AChE in different molecular forms is well established. In human brain, the total AChE levels and the distribution of its molecular forms vary regionally. The most abundant form found in human brain is the tetrameric G4, while the monomeric G1is present in smaller amounts. During aging and more dramatically in AD, the G4 form is decreased in neocortex and hippocampus, with much smaller decreases of the G1 form are found. This results in an increased enzyme activity ratio G4/G1 in those brain regions in which the severest degeneration in AD occurs. Inhibition of G1 and G4 enzyme isolated post mortem from cortex and hippocampus of AD patients was determined for rivastigmine and other AchE-l's. Rivastigmine is 4 and 6 times more potent in inhibiting the G1 form as compared to the G4 form extracted from cortex and hippocampus, respectively. In contrast, the inhibitory influence of the first generation inhibitors on the G1 and the G4 form of AChE is equipotent. The membrane-bound G4 form is located presynaptically at cholinergic nerve terminals. It seems therefore that the loss of G4 reflects the state of degeneration of cholinergic terminals in AD. On the other hand, the activity of the G1 form, inactivating ACh unrelated to release remains, unchanged. A preferential inhibition of this enzyme could be beneficial in situations of cholinergic hypofunction. There is evidence that the G1 form is complexed with beta-amyloid (Ab) found in the amyloid plaques.

Unlike donepezil which is a selective inhibitor of AChE, rivastigmine also inhibits butyrylcholinesterase (BChE). This enzyme is only marginally present in normal human brain. However in AD brains the activity of BChE is increased. New results indicate that BchE becomes associated mainly with the amyloid plaques at approximately the time when the Ab deposits assume a compact b-pleated conformation. Therefore BChE may participate in the transformation of the initially benign form of Ab to the malign form associated with neuritic tissue degeneration. Thus, ChE inhibitors like rivastigmine could potentially interact with and affect the formation of amyloid plaques.

For the time being AchE-l's are the most successful agents for the symptomatic treatment of AD. Clinical results with inhibitors of the second generation, such as rivastigmine, indicate that the disadvantages of first generation drugs might be overcome by improving CNS selectivity and thereby decreasing the peripheral cholinergic effects and toxicity. The

possibility that certain cholinesterase inhibitors might interact with the process of Ab formation suggests a potential beneficial influence of these drugs on the progression of the disease. This will open a new avenue for a causative treatment of AD in the future.

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No Neuronal Degeneration, No Alzheimer Disease

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We hypothesize that Alzheimer disease (AD) is a metabolic disorder of the mid to old age which requires a certain genetic predisposition, and one or more environmental factors. Independent of the etiology AD is characterized histopathologically by the intraneuronal accumulation of paired helical filaments (PHF), forming neurofibrillary tangles, neuropil threads and dystrophic neurites surrounding the extracellular deposits of beta-amyloid, the second major lesion. The clinical expression of AD correlates with the presence of neurofibrillary degeneration; beta-amyloid alone does not produce the disease clinically. Thus, arresting neurofibrillary degeneration offers a promising key target for therapeutic intervention of AD.

The major protein subunit of PHF is the microtubuleassociated protein tau (Grundke-lqbal et al., J. Biol. Chem. 261:6084-6089,1986). Tau in AD brain, especially PHF, is abnormally hyperphosphorylated (Grundke-lqbal et al., Proc. Natl. Acad. Sci. USA, 83:4913-4917, 1986; Iqbal et al., Lancet, 2:421-426,1986). The levels of tau in AD brain are several-fold greater than in control brains and this increase is in the form of abnormally phosphorylated tau (Khatoon et al., J. Neurochem. 59:750-753, 1992). The AD phosphorylated tau (AD P-tau) contains 5-9 P04 as compared to 2-3 P04 per normal tau molecule (Köpke et al., J. Biol. Chem. 268:24376-24384,1993). AD P-tau does not promote micro tubule assembly, but on dephosphorylation its microtubule promoting activity is restored to approximately that of the normal tau (Alonso et al., Proc. Natl. Acad. Sci. USA, 91:5562-5566,1994; Iqbal et al., FEBS Lett., 349:104-108, 1994; Wang et al., Mol. Brain Res., 38:200-208,1996). Furthermore, the AD P-tau sequesters normal tau, MAP1 and MAP2 and inhibits their micro tubule assembly promoting activity (Alonso et al., Nature Med., 2:783-787, 1996; Proc. Natl. Acad. Sci. USA, 94:298-303, 1997). In vitro AD P-tau can be dephosphorylated by protein phosphatases PP-2B, PP-2A and PP-1 but not PP-2C and all the three tau phosphatases are present in brain neurons (Gong et al., J. Neurochem., 62:803-806,1994; Neuroscience, 61:705-772,1994; FEBS Lett., 341:94-98,1994; Pei et al., Brain Res.,655:70-76,1994). Tau phosphatase activity is decreased by 30% in AD brain (Gong et al., J. Neurochem., 61:921-926, 1993; ibid, 65:732-738, 1995).

It is suggested (i) that a defect(s) in the protein phosphorylation/ dephosphorylation system(s) leads to hyperphosphorylation of tau; (ii) that this altered tau causes disassembly of micro tubules by sequestering normal MAPS and consequently a retrograde neuronal degeneration; and (iii) that by increasing tau phosphatase activity, it might be possible to inhibit Alzheimer neurofibrillary degeneration.

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The Effects Of ApoE Genotype On Neuronal Maintenance And Repair: Implications For Therapy Of Alzheimer's Disease

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Previous studies revealed a marked association between the allele E4 of apolipoprotein E (apoE) and Alzheimer's disease (AD) and suggested that the deleterious effects of the apoE4 allele are mediated by distinct isoform-specific effects on

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Session 2: The role of inflammation in Alzheimer's disease

Is Alzheimer Amyloid part of an Inflammatory Cascade?

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Biochemical, genetic, and epidemiological evidence indicates that inflammation is an essential part of the pathogenesis of Alzheimer's disease. We have focussed on the role that specific inflammatory molecules play in the Alzheimer pathogenic pathway. We have learned, from both in vivo and in vitro experiments in our and other labs, that several acute phase/inflammatory molecules in the brain, specifically antichymotrypsin (ACT) and apolipoprotein E (apoE) can promote the formation of the neurotoxic amyloid deposits that are the main pathological hallmark of the disease. They do this by binding directly to the Ab peptide and promoting its polymerization into amyloid filaments. Furthermore, there is a massive overproduction of ACT in affected areas of the Alzheimer brain that is evidently caused by activation of ACT mRNA synthesis in astrocytes by the inflammatory cytokine IL-1 released from activated microglia. Recently, we have extended this inflammatory cascade to include the amyloid

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- Thomas van Groen

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- Paavo Riekkinen Jr

Estrogen Induced Genes In Rat Hippocampus

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The recent description of the delay in the manifestation of Alzheimer's disease (AD) in women undergoing estrogen replacement therapy has increased the interest in the understanding of estrogen activity in the nervous system. In spite of the longstanding knowledge of estrogen receptor (ER) presence in the brain, the progress in the study of the physiological role of estrogens in this organ is still in its infancy, mainly because of the lack of suitable and specific experimental models. We have generated the SK-ER3 cell line, a human neuroblastoma cell line stably transfected with the ER alpha isoform (ER-a-) cDNA and characterized the antiproliferative and differentiative effects that were correlated

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Genetic Dissection Of The Dementias

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-Barbara Kittner

Non-Steroidal Anti-Inflammatory Drugs And Other Agents

May Delay Onset Of Alzheimer's Disease

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It is increasingly clear that genes are the major determinant of risk for Alzheimer's disease (AD). Genes predict not only who is susceptible to AD, but also when one will develop dementia symptoms. Much evidence suggests that, long before such symptoms appear, there are ongoing changes in brain, and that these changes are also determined by genes. Thus, the pathogenetic process of AD may be characterized by a continuum that includes an extended latent phase, a briefer prodromal phase, and the familiar phase of progressive symptomatic dementia. The existence of latent and prodromal phases creates a window of opportunity for prevention. Slowing the pathogenetic process at these points should delay onset of dementia symptoms.

Five classes of medicament have now been suggested to effect such a delay. Two prospective studies suggest that post-menopausal hormone replacement therapy may act in this fashion. Numerous case control studies and one prospective study argue for a protective effect of non-steroidal anti-inflammatory drugs (NSAIDs). A curious finding of the latter studies is that low-dose daily aspirin appears to have an effect almost as robust as the more potent NSAIDs -- suggesting that the relevant action of these compounds is different from classical suppression of inflammatory processes. Histamine H2 blockers may also delay symptom onset. Findings of a single published study on H2 blockers have now been replicated in a nested, population case-base study (preliminary results to be presented verbally, pending peer reviewed publication). Several studies, including two prospective designs, suggest that anti-oxidant vitamin supplements may act similarly, and the anti-oxidant flavenoid components of red wine may explain the findings from the PAQUID incidence cohort study suggesting dramatic reduction in AD incidence among regular consumers of Bordeaux wines.

These results raise new hopes for the prevention of AD, but randomized controlled trials will be needed for definitive proof of the efficacy of any of these strategies. I am hoping to volunteer for a Bordeaux wine trial, depending on the vintage and the nature of the placebo alternative (Bourgogne?)

Aricept®: A Well-Tolerated And Clinically Effective Treatment For The Symptoms Of Alzheimer's Disease - Results From Worldwide Clinical Trials

Rogers, S.L., Mohs, R., Doody, R.S. and the ARICEPT®

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Kuopio, Finland

Poster Session

P1. EFFECT OF SELECTIVE IMMUNOTOXIC LESION OF THE SEPTAL CHOLINERGIC CELLS ON HIPPOCAMPAL PLACE CELLS

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Background: Degeneration of hippocampal cholinergic innervation is one of the best documented age-related changes in the brain. Age-associated impairment of spatial memory has been accounted for by this deficit. However, recent findings with selective cholinergic immunotoxin IgG-saporin have called this concept into question.

Methods: Hippocampal place cells were recorded from young male Long-Evans rats, 5 sham-operated and 7 with immunotoxin lesions of the septal cholinergic neurons. Analysis was performed for 50 + 52 cells that had place fields in the standard recording environment, a walled cylinder with three prominent cue cards.

Results: A geometric change (cylinder-square), a complete change of the test arena, or a new task in the familiar environment resulted in rearrangement of the place fields in both groups to the same extent. The ability to maintain similar field distribution in repeated sessions in the familiar environment was also preserved in the lesioned animals.

However, the lesioned rats showed impaired ability to remap in a new environment.

Conclusions: We conclude that degeneration of cholinergic innervation of hippocampus alone does not disrupt the place fields of hippocampal cells neither in a familiar or a new environment. However, the cholinergic innervation is needed for the remapping process to occur in a new environment.

Supported by NIA AG09973, the Academy of Finland.

P2. AMYGDALOID INTRACELLULAR CORRELATES OF PHYSIOLOGICAL HIPPOCAMPAL PATTERNS IN VIVO

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[1]Department of Neuroscience and Neurology, [2]A.I.Virtanen Institute, University of Kuopio, Kuopio, Finland

Background: The amygdaloid complex projects to several portions of the hippocampus. Via these connections the emotions processed by the amygdaloid complex may modulate the memory processing in the hippocampus.

Methods: Physiological properties of the amygdaloid neurons were examined during various hippocampal EEG patterns in urethane anaesthetized rats in vivo. Amygdaloid neurons were characterized and recorded intracellularly during physiological hippocampal EEG-patterns recorded from CA1 stratum oriens. Thereafter, the cells were filled with biocytin for 3D-reconstruction.

Results: When hippocampal theta was induced by tail-pinch, the pyramidal type neurons in the basal and accessory basal nuclei depolarized (approximately 10 mV) and increased their firing rate. During non-theta stages the amygdaloid neurons were relatively silent and did not show constant correlates with hippocampal sharp waves (SPWs) or SPW related high frequency oscillations. However, during non-theta stages, the amygdaloid neurons exhibited irregular depolarizations (for 1-2 sec) and showed increased firing rate (up to 15 Hz) simultaneously with irregular slow transients in hippocampal EEG.

Conclusions: These data provide further evidence for functional relationship between the hippocampal formation and the amygdaloid complex.

P3. TRANSGENIC MICE DISPLAY NORMAL ENTORHINAL CORTEX LESION-INDUCED SPROUTING IN THE HIPPOCAMPUS

I. Kadish, Th. van Groen, P. Riekkinen Jr

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Background: It has been demonstrated by many studies in rats that, following entorhinal cortex ablations, the dentate gyrus shows an early degeneration of the lesioned axons and terminals, followed later by a sprouting response of non-lesioned axons. We hypothesized that this response would be altered in transgenic mice expressing mutant human presenilin 1 (M146L mutation). Therefore we lesioned the entorhinal cortex in these mice, and in control animals.

Methods: The entorhinal cortex was unilaterally lesioned by injections of ibotenic acid; four weeks later the animals were sacrificed and transcardially perfused. The brains were cut and stained for several markers that have been used to analyze sprouting in rats, the most consistent changes were present in the material stained for synaptophysin, a protein that marks presynaptic terminals.

Results: Following lesions of the entorhinal cortex the ipsilateral hippocampus demonstrates sprouting. The increase in expression of synaptophysin was present in the outer molecular layer of the dentate gyrus. The control, and both the normal human and the mutant presenilin expressing mice had a similar response to the lesion.

Conclusions: Therefore, neither the presence of the normal human presenilin 1 gene nor the presence of the M146L mutation in these mice changed the response of the brain to lesions compared to mice expressing the mouse presenilin 1 gene.

P4. EFFECTS OF LOCAL INFUSION OF NMDA- AND MUSCARINIC ANTAGONIST INTO THE DORSOMEDIAL AND DORSOLATERAL PREFRONTAL CORTEX OF RATS IN DELAYED NON-MATCHING TO POSITION TEST

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Background and methods: The present study investigated the role of NMDA and muscarinic acetylcholine receptors in the

dorsomedial and -lateral prefrontal cortex in the control of spatial working memory of rats. Therefore, we studied the effect of local administration of an NMDA-receptor antagonist CPP (0.1 and 0.3 microg) and a non-selective muscarinic receptor antagonist scopolamine (10 microg) into the dorsomedial and -lateral prefrontal cortex of rats on spatial working memory task (Delayed non-matching to position).

Results: Infusion into the dorsomedial prefrontal cortex: CPP 0.1 microg impaired working memory accuracy delay-dependently, and disrupted motor behavior. CPP 0.3 microg caused a delay independent memory and motor performance deficit. Scopolamine 10 microg had no effect on memory accuracy, but impaired motor function. Infusion into the dorsolateral prefrontal cortex: CPP 0.3 microg affected motor behavior, but CPP 0.1 microg and scopolamine 10 microg had no effect on behavior.

Conclusions: These results suggest that NMDA-receptors are needed for spatial working memory function in dorsomedial, but not in dorsolateral, prefrontal cortex.

Supported by University of Kuopio and Academy of Finland.

P5. ESTROGEN AND NMDA ANTAGONISM: EFFECTS UPON REFERENCE AND WORKING MEMORY

I. Wilson, J. Puoliväli, P. Riekkinen Jr

Department of Neuroscience and Neurology, University of Kuopio, Finland

Background: There is strong evidence that estrogen has a neuroprotective effect and discourages the onset of Alzheimer's Disease. Furthermore, studies have linked estrogen with improved performance by rats on spatial learning and memory tasks.

Methods: After undergoing ovariectomy or a sham operation, mice were given a 2 week recovery before behavioral tests began under the influence of (±)-3-(2-carboxypiperazin-4-yl)-propyl-1-phosphonic acid (CPP) or vehicle treatment. CPP (2, 5 and 10 mg/kg, intraperitoneally) is a competitive, full N-methyl-D-aspartate (NMDA) antagonist, able to cross the blood/brain barrier. Such an NMDA antagonist should impair performance on spatial reference and working memory. Spatial reference memory was tested by the water maze, spatial working memory was tested by the radial arm maze, while overall activity was monitored by the Y-maze.

Results: Results from the water maze and the Y-maze did not show any activity or spatial reference memory differences between sham operated and ovariectomized mice. The radial

arm maze, however, highlighted some working memory differences between sham operated and ovariectomized mice. There were no interactions between operation and the NMDA-antagonist. CPP treatment did, however, impair the performance on both water maze and radial arm maze.

Conclusions: These results suggest that short term estrogen deprivation has no effect upon spatial reference memory, while exerting some effect upon spatial working memory. This effect is probably not mediated by NMDA receptors.

P6. ALPHA2C-ADRENOCEPTOR KNOCK OUT AND DSP-4 LESION INCREASES THE SEDATIVE EFFECT OF ALPHA2-AGONIST

J. Puoliväli, M. Björklund, M. Riekkinen, J. Sallinen, M. Scheinin, A. Haapalinna, B. Kobilka, P. Riekkinen Jr

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Methods: We investigated the function of 2C-adrenoreceptors (2C-AR) in the regulation of cortical EEG activity and in the mediation of the sedative action of 2-agonist, dexmedetomidine (DEX 3-300 g/kg sc.) in female 2C-AR knock out (KO) and control (WT) mice. The effect of noradrenergic depletion (90 %) induced by using DSP-4 (N-(2-chloroethyl)-N-ethyl-2-bromobenzylamine) on cortical EEG activity was also studied.

Results: Analysis of baseline activity revealed that theta (4-8 Hz), alfa (8-12 Hz) and beta (12-20 Hz) activities were higher in KO mice compared with WT mice. DEX 30 g/kg more effectively increased delta (1-4 Hz) and decreased theta, 20-30 Hz and 30-60 Hz activity in KO than WT mice. DEX 300 g/kg decreased theta and beta activities more effectively, and 30-60 Hz activity less effectively in KO than WT mice. DEX 300 g/kg decreased theta and alfa activities more effectively in DSP-4 lesioned KO than WT mice. DEX 300 g/kg decreased beta amplitude as effectively in DSP-4 lesioned KO and WT mice compared with vehicle treated mice.

Conclusions: These results show that the sedative action of 2-agonist, dexmedetomidine, on cortical arousal is enhanced in KO mice compared with WT mice and that DSP-4 lesion enhances the sedative action of 2-agonist, especially in KO mice. This results suggest that 2C-AR is not essential for the mediation of sedative actions of 2-agonists which are more likely mediated by the postsynaptic 2A/B-ARs.

Supported by University of Kuopio, Finnish Academy of Sciences and Orion-Farmos Ltd.

P7. OVEREXPRESSION OF ALPHA2C-ADRENOCEPTORS IMPAIRS WATER MAZE NAVIGATION

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Background: We investigated the importance of stimulation of overexpressed alpha2c (a2C) -adrenoceptors (AR) by noradrenaline in the WM defect in a2C-AR overexpressing (OE) mice. OE mice swam more in the peripheral annulus of the pool and did not find a hidden escape platform as well as wild type (WT) mice. A subtype-nonselective a2-AR antagonist, atipamezole (ATI, 1000 mg/kg, s.c.), did not improve platform finding in WT mice, but fully reversed the defect in platform finding in OE mice. We have previously found that a2C-knockout (KO) mice, were less sensitive than their controls to the defect in spatial navigation induced by a subtype-nonselective a2-AR agonist, dexmedetomidine (10 mg/kg, s.c.). This finding with KO mice supports our contention that a2C-overexpression in anatomically relevant brain areas is responsible for the performance defect in OE mice. Results: Noradrenaline depletion (-95 %) induced by DSP-4 did not impair platform finding of WT or OE mice. DSP-4 lesion slightly increased swimming in the peripheral annulus in WT mice, but not in OE mice. DSP-4 lesion produced a dissociable effect on the action of ATI to improve platform finding and search strategy in OE mice: ATI did not alleviate platform finding defect in DSP-4 lesioned OE mice, but normalized abnormal search strategy of OE mice. Noradrenaline depletion (-95 %) induced by DSP-4 did not impair platform finding of WT or OE mice. DSP-4 lesion slightly increased swimming in the peripheral annulus in WT mice, but not in OE mice. DSP-4 lesion produced a dissociable effect on the action of ATI to improve platform finding and search strategy in OE mice. ATI did not alleviate platform finding defect in DSP-4 lesioned OE mice, but normalized abnormal search strategy of OE mice.

Conclusions: These results suggests that abnormal search pattern and defect in the accuracy of platform finding are mediated by constitutive activity of overexpressed a2C-ARs.

P8. THE ROLE OF THE ESTROGEN REPLACEMENT THERAPY IN LEARNING AND MEMORY IN FEMALE MICE

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Background: Estrogen replacement therapy (ERT) seems to play an important role in preventing Alzheimer's disease, however there is a lack of information about ERT in mice. In order to study ERT and its possible effects on AlzheimerÆs disease animal models i.e., transgenic mice, we needed to study normal female mice. In this study we investigated whether ERT has an effect on learning and memory.

Methods: Female mice were ovariectomized and given estrogen or placebo treatment. ERT was given by placing an estrogen pellet under the skin of the mouse, there were three testing groups; low estrogen dose, high estrogen dose and vehicle without estrogen pellet. Different behavioural studies were done in order to study effects ERT might have in learning, the behavioural tasks were; Morris water maze, radial arm maze and t-maze. The weight of the uterus was used as an indicator the success of ERT. The uterus of the vehicle group animals was significantly shrunken compared to the estrogen treated groups and uterus of high and low dose estrogen treated animals was larger than normal uterus in mice.

Results: The animals in the vehicle group were impaired in all bevioural tasks. Animals who received estrogen performed better in spatial learning and also in reversal learning. Eventhough the vehicle group eventually learned as good as others they took a longer time to learn.

P9. EXPRESSION OF SEIZURE-RELATED PTZ-17 IS INDUCED BY POTASSIUM DEPRIVATION IN CEREBELLAR GRANULE CELLS

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Background: Neuronal apoptosis is considered to play a significant role in several neuropathological conditions, such as Alzheimer's disease, and Parkinson's disease. However, the molecular mechanisms underlying neuronal apoptosis are poorly understood. The aim of this study was to identify changes in gene expression during neuronal apoptosis using the differential display (DD) technique.

Methods: Potassium deprivation was used to induce neuronal apoptosis in cultured rat cerebellar granule cells (CGCs). Total RNA from potassium deprived and control cultures was

analyzed with DD technique using 24 primer combinations. The results were confermed with northern blot.

Results: DD analysis of about 1600 transcripts resulted in 8 cDNA clones that confirmed differential expression in a slot blot analysis. One of these clones was homologous to the 3' end of seizure-related PTZ-17 RNA. Northern blot analysis showed a marked upregulation of a 2.2 kb RNA 24 hours after potassium withdrawal. The increase in PTZ-17 expression was specific for potassium deprivation induced apoptosis, since the other apoptosis inducers, okadaic acid and staurosporine, did not affect PTZ-17 expression. The level of PTZ-17 RNA was not significantly affected by aging in rat cerebellum.

Conclusions: Our data suggest that the upregulation of the PTZ-17 RNA is a part of the steps leading to apoptosis during potassium deprivation in cerebellar granule cells.

P10. EXPRESSION OF TRANSCRIPTIONAL REPRESSOR PROTEIN mSin3A IS INDUCED DURING NEURONAL APOPTOSIS

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Background: Histone acetylation has a key role in transcriptional activation, whereas deacetylation of histones correlates with the transcriptional repression and silencing of genes. mSin3 proteins act as an adaptor molecules binding both transcription factors and histone deacetylases and they have an important role in transcriptional repression mediated by histone deacetylation. Our purpose was to find out whether apoptosis affects the expression of mSin3 proteins in neuroblastoma 2a cells.

Methods: Mouse neuroplastoma 2a cells were cultured and apoptosis was induced by serum withdrawal or by treatment with etoposide, okadaic acid or trichostatin A. Western blot and immunoprecipitation assays were done to detect changes in protein levels and synthesis rate of mSin3A and mSin3B proteins. Also caspase-3 activities were analyzed.

Results: All apoptosis models induced a prominent increase in mSin3A protein level, but did not affect the level of mSin3B protein.Trichostatin A, an inhibitor of histone deacetylases, induced the most prominent upregulation of mSin3A protein. Metabolic labeling and immunopresipitation of mSin3A showed a marked increase in the synthesis of mSin3A protein. Upregulation of mSin3A protein expression preceded caspase-3

activation and the execution phase of neuronal apoptosis.

Conclusions: These results suggest that the expression of mSin3A proteins may provide a regulation mechanisms to enhance transcriptional repression or silencing of genes during neuronal apoptosis, as well as during degenerative diseases.

P11. SELEGILINE PROTECTS NEURONAL CELLS AGAINST APOPTOSIS INDUCED BY PROTEIN PHOSPHATASE INHIBITOR OKADAIC ACID

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Background: Selegiline is a well-known inhibitor of MAO-B and is widely used for the treatment of Parkinson's disease. It has also neuroprotective effects in vivo and in vitro models involving apoptosis.

Methods: We examined the effect of selegiline on okadaic acid (final conc. 10-50 nM), etoposide (5-10 (M), cytosine b-D-arabinoside (1-100 (M), low potassium (5 mM) and serum deprivation -induced apoptosis in cultured rat hippocampal (HC), cerebellar granule (CGC) and mouse neuro-2a neuroblastoma cells. Cells were treated with apoptotic inducers for 24 or 48 h. Selegiline (10-13-10-5 M) was added at the same time as drugs or withdrawal of potassium or serum. Induction of apoptosis was characterized by morphological changes and by the activation of caspase-3. The viability of cultures was quantified by lactate dehydrogenase activity (LDH) of culture medium and by the tetrazolium salt (MTT) method.

Results: Selegiline decreased the activation of caspase-3 in all three types of neurons treated with okadaic acid. Also cell death was reduced in selegiline treated HC and CGC cells compared to okadaic acid treatment measured by LDH and MTT. The neuroprotective effect of selegiline was dependent on the concentration of the drug. However, selegiline did not provide neuroprotection against apoptosis induced by etoposide, cytosine b-D-arabinoside, low potassium or serum withdrawal.

Conclusions: These observations show that selegiline reduses apoptotic cell death in cultured neurons after okadaic acid treatment but not after etoposide or cytosine b-D-arabinoside treatments or potassium or serum deprivation.

P12. UPREGULATION OF UBIQUITIN-BINDING p62 IN NEURONAL APOPTOSIS

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Background: Neuronal death by apoptosis is involved in various neurodegenerative conditions such as Alzheimer's disease. The molecular mechanisms of neuronal apoptosis are still incompletely understood. We have identified genes that are down- or upregulated at the mRNA level during neuronal apoptosis in vitro using a cDNA array technique.

Methods: Apoptosis was induced in Neuro-2a cell cultures by serum deprivation or treatment with anisomycin, etoposide, okadaic acid, or staurosporine. For the array hybridization experiment, apoptosis was induced by serum deprivation, and total RNA samples were extracted from apoptotic and control cultures. Atlas mouse cDNA arrays (Clontech) were hybridized using cDNA probes prepared from the RNA samples. Temporal expression of p62 mRNA during apoptosis induced by various treatments was analyzed by Northern blot.

Results: One of the mRNAs identified encodes a 62 kD protein previously shown to interact with ubiquitin, p56lck protein kinase, and PKC-d. In all apoptotic treatments tested, p62 mRNA is markedly upregulated, the temporal pattern of upregulation depending on the type of treatment. The most prominent increases of p62 mRNA are seen in okadaic acid treated and serum deprived cultures.

Conclusions: The p62 gene is an immediate early response gene encoding a protein of unknown function. However, according to recent studies, the p62 protein likely regulates fates of ubiquitinated proteins in a signal-dependent manner. Our data therefore suggests that p62-regulated proteolytic pathways may have a significant role in neuronal apoptosis.

P13. BETA-AMYLOID (1-42) AFFECTS INTRACELLULAR MTT TRAFFICKING BUT DOES NOT INTERFERE WITH ENERGY METABOLISM OR DIMINISH PROLIFERATIVE ACTIVITY OF CULTURED RAT ASTROCYTES

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Background: Beta-amyloid (Ab) peptide deposition in the brains of Alzheimer's disease patients results in reactive astrogliosis that may contribute to neuronal cell death. Ab has also been reported to impair important supportive astrocyte functions such as glutamate uptake in vitro.

Methods: We studied the effect of different amyloid peptides on astrocyte 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) reduction that is assumed to reflect cell redox activity. The effect of Ab(1-42) peptide on cellular ATP content, lactate release and proliferation were further characterized.

Results: Ab(1-42) decreased astrocyte MTT reduction significantly, starting at nanomolar levels, in the absence of cell death, whereas Ab(1-40) was effective only in aggregated form and at high concentrations. At first the Ab treated cells displayed normal initial rate of MTT reduction. However, after 15 min incubation the cells started to produce extracellular crystals of MTT and seized to reduce MTT associated with cell death. No significant effects of Ab(1-42) on cellular ATP levels and lactate release were found. The cells displayed increased proliferation after treatment with Ab(1-42) but not with Ab(1-40).

Conclusions: Our results do not support the existence of major energy metabolism changes or energy depletion in Ab treated astrocytes. The effect of Ab peptides on MTT reduction in astrocytes may be due to enhanced removal of MTT from the cells as suggested by accumulation of extracellular MTT crystals. We conclude that aggregated amyloid peptides affect the intracellular transport machinery in astrocytes, and that this change may disturb the function of astrocytes in Alzheimer's disease brain.

P14. CONSTRUCTION OF A MODEL SYSTEM FOR AMYLOIDOGENIC STUDIES

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Amyloids are a group of biochemically heterogeneous entities of which each type is associated with a particular disease. Although the amino acid sequence of several amyloid forming proteins is different, we have recently found that most of the amyloidogenic peptides contain a fragment with two common characteristics. There seems to be a hydrophobic cluster containing 4-6 hydrophobic amino acids in a sequence of approximately 6 residues and this sequence is a part of a bigger fragment which is predicted to have a high potential for beta-

sheet conformation. We proposed that any peptide sequence with the characteristics mentioned above will form amyloid-like fibrils when exposed to proper environmental factors.

In order to study the common mechanism underlying the fibril formation we have constructed artificial amyloidogenic proteins, which have interchangeable amyloidogenic core sequences. These hybrid proteins are expressed in E. coli. Our initial model is based on gelsolin, the protein that is the origin of fibril forming fragments found in the amyloidosis of Finnish type (FAF). Using PCR-technique we have deleted the sequence responsible for the amyloid fibril formation in this protein and replaced it with several distinct amyloidogenic sequences.

The aim of this study is to determine whether these sequences from different amyloid proteins are able to form amyloid fibrils, and which are the factors influencing these structural changes.

P15. MORPHOMETRICAL EVALUATION OF HUMAN BRAIN VASCULARIZATION - COMPARISON OF MANUAL AND AUTOMATIC METHODS.

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Background: Pathological changes in the brain vascularization and human blood-brain barrier function may contribute to the pathogenesis of Alzheimer's disease. Morphometry is one of the most often used methods to evaluate the brain vascularization. Many different morphometrical methods can be applied in such studies - both modern, computer-based automatic methods as well as traditional, manual methods, still in use. In the present study we compared results obtained using modern and traditional method.

Methods: Post-mortem material from 5 unimpaired individuals without known neurological disorder were analyzed. Vessels were visualized in varied brain regions (frontal, temporal, and parietal cortices) employing immunohistochemical staining (monoclonal HaM 56 antibody, DAKO M632). The vessel density and the stained area fraction were estimated morphometrically using Quantimet 570 and Weibel's grid.

Results and conclusions: The results obtained applying automatic and manual methods were comparable even though the values were slightly higher using automatic method. Our findings indicate that automatic methods which are fast and

reproducible are recommended specifically when large material is to be analyzed.

Preliminary results indicate that both the vessel density and the stained area fraction were smaller in the white matter compared to the gray matter and the vascular density was lowest in temporal cortex.

P16. THE MORPHOLOGY OF THE SPINY NEURONS OF THE HUMAN ENTORHINAL CORTEX: A SINGLE CELL INJECTION STUDY

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Background: There are several studies concerning the morphologies of the primary neurons of the entorhinal cortex (EC) in rodents and primates. In human, such studies are scarce, although morphological studies are important for understanding the function as well as dysfunction of EC.

Methods: We studied the morphological differences of spiny primary neurons in layers II, III, V and VI of the eight subfields of the human EC using Lucifer yellow microinjection.

Results and Conclusions: We were able to differentiate four types of spiny neurons according to the shape of the somata and primary dendritic tree: classical pyramidal, stellate; modified stellate; and horizontal tripolar cells. In layer II majority of the neurons were stellates or modified stellates. In the rostromedial subfields of the layer II also horizontal tripolar neurons were found. Layer II neurons had the densest ramification of the dendritic tree in the cellular cluster of layer II. In the layers III, V and VI the neurons were mainly classical pyramids, being the smallest in somal size in layer III. Our study reveals that there are at least four morphologically different types of primary neurons, which have specific laminar organization in the human EC.

P17. HIGHLY POLYSIALYLATED NEURAL CELL ADHESION MOLECULE (PSA-NCAM) EXPRESSION IN THE ENTORHINAL CORTEX AND THE HIPPOCAMPUS: REMODELING OF NEURONAL CIRCUITRIES IN ALZHEIMER'S DISEASE AND TEMPORAL LOBE EPILEPSY

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Background: PSA-NCAM is mainly expressed in embryonal nervous system. In adult brain it is associated with continuous growth, repair of tissue, and generation of neurons.

Methods: We studied PSA-NCAM immunoreactivity (-ir) in the dentate gyrus (DG) and entorhinal cortex (EC) of controls, and in temporal lobe epilepsy (TLE), or Alzheimer's disease (AD).

Results and Conclusions: In controls, the hilus of DG contained numerous PSA-NCAM-ir. Occasional PSA-NCAM-ir infragranule cells were located in the innermost portion of granule cell layer. The outer two-thirds of the molecular layer (oml) was more intensely labelled than inner third of the molecular layer (iml). In EC, radially oriented fiber bundles were located in layers II and III. In TLE, the density of PSA-NCAM-ir neurons in the hilus was decreased. Preserved neurons had tortuous dendrites in proximity of somata. In eight cases with mild neuronal loss of hippocampus, the density of infragranule cells was higher compared to controls. Other cases had only occasional infragranule cells. The lamination of oml and iml was diminished due to the substantial increase in PSA-NCAM-ir of iml. The rostromedial EC showed abnormal plexi of fibers surrounding small neurons in layer II. In AD, the hilus contained morphologically similar neurons as in controls. The density of hilar and infragranule cells was preserved. The staining intensity of oml was increased. In EC non-oriented fibers were seen in layer II. Differences in PSA-NCAM-ir between controls and TLE or AD suggests that neuronal remodeling occurs in both diseases.

P18. DISTRIBUTION OF MUSCARINIC (m2) ACETYLCHOLINE RECEPTORS IN THE RAT ENTORHINAL CORTEX

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Background: There is convincing evidence about the hypofunction of cholinergic system in Alzheimer's disease and a number of cholinergic replacement therapies, has been

developed. However, the mechanisms underlying the cholinergic neurotransmission is still poorly understood. For example, the neuronal circuitries carrying different types of acetylcholine receptors are largely unknown. Therefore, in the present study, we examined the distribution of muscarinic (m2) acetylcholine receptors in the entorhinal cortex which is highly vulnerable in Alzheimer's disease and degeneration of which is associated with cognitive deficits.

Methods: We utilized light and confocal microscopy to study distribution of m2 receptors and to identify and characterize the neuron types expressing these receptors.

Results: The analyses revealed that the rat entorhinal cortex exhibite a high number of m2 receptors. In addition, they are relatively homogeneously distributed in different layers and subfields, only the layer I showing the lightest intensity. These receptors are scattered diffusely and only seldomly an individual cell body immunoreactive for m2 receptors can be noticed. Accordingly, our preliminary analysis revealed no parvalbumin, calretinin, or somatostatin-containing cells which contained m2 receptors in their somata.

Conclusions: These findings suggest that the muscarinic m2 acetylcholine receptors operate in the dendritic regions either pre- or postsynaptically. Furthermore, being highly expressed in the entorhinal cortex, muscarinic (m2) acetylcholine receptors are likely to have a crucial role in information processing in this brain area.

P19. CHOLINERGIC NEURONS IN THE RAT BASAL FOREBRAIN EXPRESS ESTROGEN RECEPTOR ALPHA SUBTYPE

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Background: The basal forebrain area constitutes a heterogeneous collection of cell groups, which are involved in a variety of physiological and behavioral processes. Cholinergic cells are the major projection neurons of this region and they give rise to widespread efferents to entire cortical mantle. Degeneration of these neurons is associated with impairment of higher cognitive functions which is typical for Alzheimer's disease. Epidemiological studies have suggested that estrogen replacement therapy has protective effect against Alzheimer's disease. There is evidence that this effect is mediated via estrogen's interaction with cholinergic system. In fact, distribution of the cholinergic cells overlap with that of cells expressing estrogen receptors. Therefore, in the present study

we investigated whether the cholinergic cells in the basal forebrain contain estrogen receptor a. **Methods:** The colocalization analyses was carried out on rat brain sections which were double immunostained for choline acetyltransferase and estrogen receptor alpha. **Results:** Our preliminary analyses revealed that 94.5 % (208 out of 220) of all cholinergic neurons in the medial septum, 87.7 % (157 out of 179) in the basal nucleus of Meynert, and 97.8 % (566 out of 579) in the diagonal band contain estrogen receptor alpha. **Conclusions:** These results provide anatomical basis for the interaction of estrogen and cholinergic system suggesting that estrogen modulates directly the activity, and most likely different transcriptional factors, of the cholinergic neurons.

P20. SUBFIELD- AND LAYER-SPECIFIC DISTRIBUTION OF ESTROGEN RECEPTOR ALPHA SUBTYPE IN THE RAT ENTORHINAL CORTEX

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Background: The entorhinal cortex is a central element of hippocampal and neocortical interconnectivity. Information between the neocortex and hippocampus is processed in different layers and subfields of the entorhinal cortex in a highly topographically organized manner. The knowledge of neuronal circuitries and involvement of different receptors in these pathways are of crucial importance for the understanding of pathophysiological processes of different diseases associated with destruction of the entorhinal cortex. Since estrogen has recently shown some promise in the treatment of Alzheimer's disease, the present study was undertaken to investigate the distribution of estrogen receptors in the entorhinal cortex.

Methods: Immunohistochemistry against estrogen receptor alpha was utilized on the sections of rat entorhinal cortex.

Results: The analyses revealed that cells expressing estrogen receptor alpha are mostly located in layers V and VI of the dorsal and ventral intermediate subfield. Layer III of these subfields as well as the amygdalo-entorhinal transitional subfield also contain relatively high number of these receptors. However, layers I and II of each subfield and all layers of caudal, medial and dorsal lateral subfield are devoid of them.

Conclusions: These results indicate that the estrogen receptor alpha subtype is specifically involved in those circuitries which relay the information that has already been processed by the hippocampus and which will be forwarded back to the neocortex, probably for long term storage.

P21. ABNORMAL SYNAPSES IN THE CEREBELLAR CORTEX IN ALZHEIMER'S DISEASE: A GOLGI AND ELECTRON MICROSCOPE STUDY

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Background: Neuritic plaques and neurofibrillary tangles are the morphological hallmarks of Alzheimer's disease, seen mostly in the hippocampus and the cortex of the cerebral hemispheres.

Methods: This morphological study is based on examination of ten brains obtained at autopsy 30 min. after death. Samples from the vermis and the hemispheres of the cerebellum were excised and immediately immersed in Sotelo's fixing solution and they were processed for electron microscope .The brains, which were processed for the silver impregnation techniques, wereremained for two weeks in formalin. Part of the vermis and the cerebellar hemispheres were excised and immersed in potassium dichromat (7g potassium dichromat in 300 ml water) for ten days. Then the specimens were immersed in 1% silver nitrate for ten days, according the rapid Golgi method.

Results: In the cerebellum, the morphological analysis, revealed limited number of neuritic plaques and numerous synaptic alterations concerning the parallel fibres and the Purkinje cell dendritic spines as well the climbing fibres and the Purkinje cell dendrites. In the granule layer, the number of the granule and Golgi cells was impressively decreased, in correlation with normal controls. The synapses between the mossy fibres and the granule cell dendrites were also decreased. Some of the synapses, that remained still intact, contained limited number of polymorphic synaptic vesicles and numerous morphologically alternated mitochondria and dense bodies in the mossy fibre presynaptic terminals. The number of synaptic contacts between the mossy fibre terminals and the dendrites of the granule and Golgi cells was dramatically decreased. In the molecular layer substantial loss of stellate and basket cells was noticed as well as numerous synaptic alterations between parallel fibers and Purkinje cell dendritic spines.

Conclusions: The above described observations, plead obviously in favour of neuronal loss and synaptic alterations, as a primary cause of cerebellar dysfunction in Alzheimer's disease.

P22. PROMOTER POLYMORPHISM (-491A/T) IN THE APOE GENE OF FINNISH ALZHEIMER'S DISEASE

PATIENTS AND CONTROL INDIVIDUALS

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Background: Apolipoprotein E (APOE) e4 allele is a major risk factor for the development of Alzheimer's disease (AD). However, it has been suggested that the quantitative expression of APOE alleles results from mutations in the promoter region of this gene.

Methods: In this study, we studied the -491 A/T promoter polymorphism and whether it was dependent on the APOE e4 allele using clinic-based AD (n=106) and community-based control (n=123) samples. The -491 A/T and APOE polymorphisms were analysed using the polymerase chain reaction (PCR) method and restriction fragment length polymorphism analysis.

Results: The APOE e4 allele was strongly associated with AD when compared to controls. p(c2)<0.001 (odds ratio 5.85, 95 % Cl 3.29-10.41). However, the genotype distribution of the -491 A/T polymorphism did not significantly differ between the study groups (P=0.063), and the 491 A allele was not associated with a significant risk in the AD group when compared to controls (odds ratio 1.82, 95 % Cl 0.95-3.49). Furthermore, the 491 A/T polymorphism was independent of APOE e4 allele in both AD cases and controls (P=0.916), although linkage disequilibrium between the APOE and the -491 A/T polymorphism was associated with the -491 A allele.

Conclusions: Therefore, APOE polymorphism remains the most efficient predictor of risk in AD.

P23. LINKAGE DISEQUILIBRIUM BETWEEN MICROSATELLITE MARKERS AT 13q12 REGION IN FINNISH LATE-ONSET ALZHEIMER'S DISEASE PATIENTS.

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Background: Alzheimer's disease (AD) is a complex

neurodegenerative disorder, which comprises of several disease-associated loci in different chromosomes. Genes contributing to late onset AD have been suggested to exist in a number of chromosomes, such as in chromosome 13. We have used a population based linkage disequilibrium mapping approach in order to find the potential AD-associated loci from chromosome 13.

Methods: Chromosome 13-specific microsatellite markers were PCR amplified and the sizes of PCR products were determined with automated fragment analyser.

Results: During the screening with chromosome 13-specific microsatellite markers, adjacent markers D13S292 and D13S787 were found to be in linkage disequilibrium at the 13q12 region. Stratification of the AD patients and controls into the groups according to the apolipoprotein E, sex and familial/sporadic status indicated that 13q12 locus was associated with female familial AD patients regardless of ApoE genotype. Based on the physical data obtained from the region 13q12, markers D13S292 and D13S787 were estimated to reside in a 810 kb long YAC clone 754h7 together with two infant brain-derived ESTs and the H,K-ATPase (-subunit protein gene (ATP1AL1).

Conclusions: The fact that the ATP1AL1 and the ESTs are located at the linkage disequilibrium region, suggests that one of these genes may have a role in late onset AD.

P24. APOE PHENOTYPE DOES NOT INFLUENCE SURVIVAL IN ALZHEIMER'S DISEASE IN POPULATION BASED LONGITUDINAL STUDY

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Background: Apolipoprotein E (4 (apoE4) is a known risk factor for Alzheimer's disease (AD). However, the effect of ApoE4 on survival in AD is controversial. Our aim was to investigate survival in AD patients by ApoE4 and sex.

Methods: A random sample of 1299 subjects (65-74 years in baseline) were drawn from register of Kuopio. Cognitive evaluation was performed for totally 1192 subjects. Forty eight cases with AD were identified. The follow up period was seven years for the whole study group and nine years for the AD patients.

Results: ApoE4 was more frequently present in AD patients (56%) than in controls (32%). In the whole study population survival was not associated with AD or ApoE4. Instead, men had higher mortality than women independently of ApoE phenotype (HR 0.5, 95% confidence interval 0.44 to 0.69). In AD patients survival was not influenced by ApoE phenotype or sex when analysed separately. An interaction between ApoE4 and sex was found. In patients with AD men not carrying ApoE4 had higher mortality than the respective women ($p<0.01$).

Conclusions: In the aged population ApoE4 or AD does not influence mortality while risk of death is increased in men. Once AD has manifested, ApoE4 per se does not increase mortality. However, in the AD patients not having ApoE4 men have reduced survival compared to women.

P25. COGNITIVE DYSFUNCTION IN NON-DEMENTED TYPE-2 DIABETES PATIENTS? A POPULATION STUDY

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Background: Non-insulin-dependent diabetes mellitus (NIDDM) has been associated frequently with impaired memory. Large population studies have reported an association between NIDDM and Alzheimer's disease, suggesting that cognitive dysfunction could be solely due to primary dementia. However, no population-based studies excluding demented patients have been reported. Therefore, we studied cognitive function in an elderly non-demented population.

Methods: Cognitive function and glucose tolerance was measured in 980 subjects aged 69-78 years, of whom 915 were non-demented. Cognitive testbattery included: Buschke Selective Reminding Test, Visual Retention Test, Trail-Making Test parts A (TM-A) and C (TM-C), Verbal Fluency Test and Mini-Mental State Examination (MMSE).

Results: Patients with NIDDM were impaired in the TM-A and TM-C compared to the non-diabetic subjects. When sexes were studied separately, men were unimpaired, whereas women were impaired in the TM-A and TM-C, however, they scored better than non-diabetic women in the MMSE. Time to complete TM-A and TM-C may reflect to a great extent non-cognitive motor and visual scanning problems.Therefore, a difference score (TM-C-TM-A) reflecting "true" processing time may be calculated. Difference score was slightly elevated only in

diabetic women.

Conclusions: We conclude that in a non-demented population, cognitive dysfunction is not present in men with NIDDM, whereas in women slightly slowed information processing may be found. Most importantly, memory impaiment was not present. Primary degenerative dementias may affect cognitive function in NIDDM, but NIDDM per se is not a disorder impairing memory.

P26. CHANGES ON MEMORY FUNCTIONS AND MRI VOLUMETRY IN AAMI SUBJECTS AND HEALTHY ELDERLY SUBJECTS: A FOLLOW-UP STUDY

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Background: Several studies have indicated that mild cognitive impairment does have value for detecting early preclinical dementia. Hippocampal atrophy detected by volumetric MRI is a sensitive feature early in the course of Alzheimer's disease. The aim of this study was to evaluate changes on memory functions and MRI-based hippocampal volumetry in AAMI subjects and cognitively healthy elderly subjects during a follow-up period of 2.8 years.

Methods: We investigated 15 AAMI subjects, using the original diagnostic criteria for AAMI, and 18 age-matched elderly subjects. Three neuropsychological tests were used to assess memory functions: Buschke Selective Reminding Test, total recall (BSRT), Visual Reproduction Test, immediate recall (VRI) and delayed recall (VRD). A 1.5 T MRI imager was used for hippocampal volume measurements.

Results: Hippocampal volumes significantly decreased in the both groups during the 2.8-years follow-up period. The subjects with AAMI showed a significant decline in scores in the BSRT sensitive to episodic memory whereas the control subjects did not show any significant deterioration. None of the subjects were demented.

Conclusions: In our study AAMI subjects showed a progression of the memory impairment. However, a volume loss of the hippocampus occurred at a similar rate in the both groups. Continued follow-up of the participants in this study will reveal which subjects will develop AD.

P27. FUNCTIONAL MAGNETIC RESONANCE

IMAGING DURING A VERBAL FLUENCY TASK ON CATEGORY

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Background: Verbal fluency on category is a sensitive test in detecting the early stages of Alzheimer's disease (AD). Therefore, we wanted to develop a functional magnetic resonance imaging (fMRI) method to determine the distribution of neural activation during performance of verbal fluency test (VFT).

Methods: The activation paradigm was a block-designed modification of the clinical VFT on animal category. During the activation block, young volunteers (n=10; six male, four female) covertly produced items on given category, whereas during the baseline block they produced numbers. fMRI was performed with a 1.5 T scanner using a gradient-echo echo-planar imaging sequence.

Results: Compared with the producing of numbers, VFT on category resulted in activations in Broca's area and in left prefrontal cortex in all subjects. In addition, left posterior parahippocampal gyrus near fusiform gyrus was activated in 7 out of 10 subjects.

Conclusions: Our modification of VFT on category resulted in activations in Broca's area and in left prefrontal and posterior parahippocampal cortex. We will continue to compare these imaging findings with those of probable AD patients known to be impaired in this neuropsychological test.

P28. CEREBROSPINAL FLUID, BUT NOT SERUM Ab42 CONCENTRATION IS DECREASED IN ALZHEIMER'S DISEASE

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Background: Formation of diffuse and mature amyloid plaques is a characteristic feature in Alheimer's disease (AD). Soluble forms of beta-amyloid, sAb42 and sAb40, are found from CSF and blood. AD related mutations increase the production of the long form bA(42) in vivo and in vitro. CSF sAb measurement might help the antemortem diagnosis of AD.

Methods: We measured CSF and serum concentrations of sAb42 and sAb40 from 41 patients with AD (mean age 71 years), from 30 old controls (65 years) and 12 young controls (26 years) and serum concentrations from 30 Down's syndrome patients (48 years) using an ELISA assay.

Results: There was a significant decrease of CSF sAb42 levels in AD compared to those of old controls (median 39 vs 120 pg/ml Kruskall-Wallis $p<0.005$). Levels of CSF sAb40 did not differ between AD patients and controls (median 11.2 vs 9.9 ng/ml). There was no correlation between age and CSF sAb42 or sAb40 concentrations. Serum levels of sAb42 and sAb40 were increased in patients with Down's syndrome compared to those of AD patients and controls (median 380, 200 and 170 pg/ml for sAb40, and 250, 0 and 0 pg/ml for sAb42).

Conclusions: Low levels of sAb42 in CSF may be due to enhanced binding into the plaques. Because of an overlap of CSF sAb42 levels, it is not useful as a diagnostic marker for AD. However, in combination with other markers it may be used for diagnosis or monitoring progression of AD.

International Project Team, Tokyo JAPAN, New York, NY, USA and Houston, Texas, USA

ARICEPT® (donepezil hydrochloride or E2020), is a piperidine-based molecule that is a potent, highly selective and reversible inhibitor of the enzyme acetylcholinesterase. ARICEPT® inhibits the activity acetylcholinesterase preferentially in brain tissue. This, in turn, produces significant increases in brain acetylcholine concentrations (measured ex vivo and by in vivo micro dialysis) and improved performance on tests of learning and memory. ARICEPT® is the most widely approved and used treatment for Alzheimer's Disease worldwide.

A comprehensive clinical trials pro gram was conducted in approximately 2000 patients from North America, Europe, Africa, Australia and New Zealand. These double-blind, placebo-controlled studies consistently demonstrated that ARICEPT®, at doses of either 5 or 10 mg, given once daily, produces highly statistically significant improvements in both cognitive (Alzheimer's Disease Assessment Scale, cognitive sub scale) and global [Clinician's Interview-based Impression of Change with Caregiver Information (ADSC-CGIC version)] function. Clinical trials have demonstrated that these benefits occur much faster with ARICEPT® than with other agents as only Aricept has demonstrated statistically significant improvement in cognitive tests as early as 3 weeks and statistically significant improvements in global function as early as 6 weeks. These benefits are achieved in the absence of hepatoxicity or intolerable gastrointestinal side effects and without the need for dose titration. ARICEPT® administration is associated with a relatively low percentage of cholinergically-mediated treatment emergent adverse events, such as diarrhea, nausea and vomiting. These symptoms, when they occur, are generally mild and transient, usually resolving within 1 to 3 days, without the need for dose modification. Long-term investigations have demonstrated that the treatment benefits of ARICEPT® are evident for more than 240 weeks (4.5 years) and that the size of the treatment effect (in comparison with untreated patients) becomes larger over time. The exact mechanism for this important benefit remains under investigation, however serial PET scans taken from patients during a 6-month double-blind clinical trial demonstrated that ARICEPT® preserved functional brain activity (as defined by glucose utilization). Additionally, data from serial MRS scans conducted on patients during a separate double-blind, placebo-controlled trial suggest that ARICEPT® attenuated the reductions in N-acetyl aspartate that have been associated with neuronal decline. These results combined with independent findings by Doody et al. and Giacobini, et al. suggest that treatment with cholinesterase inhibitors may serve to attenuate disease progression.

Metrifonate As A New Treatment For Alzheimer's Disease - Clinical Profile

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Of the numerous compounds in development for the treatment of Alzheimer's disease (AD), the acetylcholinesterase (AChE) inhibitors are the most clinically advanced. Although these compounds share a common mechanism of action, they are pharmacologically heterogeneous and hence efficacy and tolerability vary greatly within this class.

Metrifonate is a long-acting AchE-inhibitor developed by Bayer for the treatment of mild to moderately severe AD. The pharmacological attributes of this cholinesterase inhibitor make it ideally suited to restoring the cholinergic deficit observed in AD. Metrifonate is a pro-drug that is non-enzymatically converted to an active metabolite, dimethyl 2,2-dichlorovinyl phosphate (DDVP).

1. The DDVP molecule forms a stable complex with the active site of cholinesterase, providing long-lasting enzyme inhibition which by far outlasts the presence of the unbound drug in the body.

2. This allows for a convenient once-daily dosing regimen, and optimises tolerability as fluctuations in enzyme activity are practically abolished. Repeated dosing results in a gradual increase in AChE inhibition toward target levels

3. In addition, as metrifonate undergoes minimal protein binding (<15%) and is not metabolised by the hepatic cytochrome P450 system, the risk of drugdrug interactions between metrifonate and concomitant medications is low, an important feature for any therapy that will be used in an elderly population.

Clinical trials conducted to date show that these pharmacological attributes do indeed translate into significant benefits across all four key symptom domains of AD cognition, behavioural and psychiatric disturbances, activities of daily living and global function. In addition, metrifonate also significantly reduces caregiver burden, reinforcing the relevance of the benefits this treatment provides to the AD patient.

To further investigate the effect of treatment on these four domains, data were pooled from pivotal Phase III trials. These data, as well as the results from the caregiver burden assessments, are presented here. The domains cognition, global function and activities of daily living were analysed using pooled intent-to-treat, last observation carried forward (ITT-

LOCF) data from three Phase III trials - 0114,5 0115 [data on file, Bayer AG] and 0122.4 The analysis of behavioural and psychiatric disturbances used pooled data from two of these studies - 0114 and 0122. The three trials were similar in design - consisting of a 2-week screening period, followed by 26 weeks of double-blind treatment and, more importantly, all obtained similar results on individual assessment scales. The following treatment regimens were compared with placebo: Study 0114 3060 mg/day; Study 0115 40 or 50 mg/day (dosed by body weight) and 60 or 80 mg/day (dosed by body weight); Study 0122 50 mg/day. For the purposes of the analysis, patients who completed double-blind treatment in the three trials were divided into two treatment groups: those who received a lower dose of 3060 mg/day and those who received a higher dose of 60/80 mg/day.

Both dose levels of metrifonate significantly improved cognition and global function from Week 12 through to Week 26. Importantly, on both the Alzheimer|s Disease Assessment Scale - cognitive subscale (ADAS-cog) and the Mini Mental State Examination, the higher dose significantly improved cognition not only compared with placebo, but also compared with pre-treatment (baseline) levels. On the ADAS-cog the treatment difference between higher-dose metrifonate and placebo reached 3.81 points (P=0.0001) and the difference between higher-dose metrifonate and baseline levels reached 2.21 points (P=0.0001).

Metrifonate maintained the patients ability to perform instrumental and basic ADLs, whereas those receiving placebo deteriorated significantly during the 26-week treatment period, as assessed on the Disability Assessment for Dementia scale (P<0.05 between active treatment groups and placebo). In addition, both dose levels significantly improved the patients ability to initiate and plan/organize the activities compared with placebo. Patients treated with higher-dose metrifonate also showed significant improvement in their ability to execute these tasks effectively. At 26 weeks, assessments conducted on the Neuropsychiatric Inventory (NPI) show that metrifonate significantly improved the majority of the behavioural and psychiatric criteria analysed. These differences were statistically significant for the Total NPI score and for the sub-items Hallucinations, Agitation/Aggression, Depression/Dysphoria, Apathy and Aberrant Motor Behaviours.

Caregiver burden was assessed in Study 0115, the Metrifonate in Alzheimer's Trial (MALT), using several, well-validated, independent measures. Treatment with metrifonate (pooled doses) for 26 weeks significantly reduced the psychological burden of caring, compared with placebo, as measured by the Screen for Caregiver Burden-subjective scale (P=0.045), the Poulshock and Deimling-cognitive subscale (P=0.0004) and the abridged Relatives*, Stress Scale (P=0.036). Furthermore, the

caregiver|s subjective impression of the change in time spent providing care indicated that metrifonate reduced overall time spent caring, compared with placebo (P=0.044).

In conclusion, metrifonate is the first AChE inhibitor to demonstrate significant benefits across all key symptom domains of AD - cognition, behavioural and psychiatric disturbances, activities of daily living and global function *) in prospective, placebo-controlled trials. Importantly, these improvements do translate into significant benefits for the caregiver, and metrifonate is, once again, the first in its class to demonstrate such benefits in a well-designed trial. Metrifonate treatment was also safe and well tolerated - pooled safety findings show that 85% of patients treated with higher-dose metrifonate completed the 26 weeks of treatment, compared with 89% of those receiving placebo. Only 6% of the higher-dose group discontinued due to adverse events, compared with 5% of those receiving placebo.

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New M1 Muscarinic Agonists - Will These Modify Only Symptoms Or Also Disease Progression Of Alzheimer's Disease (AD)?

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Background: Conceptually, M1 muscarinic agonists represent a more rational treatment of AD than the recently FDA-approved cholinesterase inhibitors. However, some muscarinic agonists showed disappointing clinical efficacy in AD. This may be due to: lack of selectivity to M1 muscarinic receptors (m1 mAChR) (e.g. milameline); low intrinsic activity (e.g. Lu-25-109, very weak M1 agonistic activity); very low bioavailability and extensive metabolism (e.g. xanomeline, M4> M1 agonist); and narrow safety margin (e.g. all these drugs and sabcomeline). Thus, since these drugs have major clinical limitations, the proof of clinical concept could not be tested properly.

A unifying hypothesis: A cholinergic hypofunction in AD can

be tied with formation of neurotoxic (b-amyloids and this can further decrease acetylcholine (ACh) release (a presynaptic effect) and impair the coupling of m1 mAChR with G-proteins (a post-synaptic effect). This uncoupling leads to decreased signal transductions, impairments in cognition, a reduction in levels of trophic secreted amyloid precursor proteins (APPs), generation of more neurotoxic (-amyloids and a further decrease in ACh release. At least three ôvicious cyclesö (two due to (-amyloids, and a third to hyperphosphorylation of tau proteins) may be prevented, in principle, by M1 selective agonists (Fisher, Exp. Opin. Invest. Drugs 6:1395, 97).

Results: Reported M1 selective muscarinic agonists include the AF series [e.g. AF102B, AF150(S)] and talsaclidine. The AF series are rigid analogs of ACh. This rigidity limits the conformational freedom in the ligand-receptor complex, which in turn may alter the affinity of m1 mAChR to form the ternary complex, i.e. agonist-receptor-G-protein. The net result is activation of only distinct sets of G-proteins (Gq/11 but not Gs), and select signal transduction pathways mediated by m1 mAChR. This might be beneficial clinically due to relevant altered signal transductions in AD. These agonists increase APPs secretion in cell cultures, rat cortical slices and primary cortical and hippocampal neurons. Our M1 agonists promote the non-amyloidogenic APPs processing pathways and exert neurotrophic activities in conjunction with some neurotrophine-dependent and -independent signal(s) (Haring et al, J. Neurochem., 71:2094, 98). Furthermore, activation of m1 mAChR leads to: 1) reduction in tau hyperphosphorylation (in vitro and in brains in vivo); 2) inhibition of apoptosis; 3) expression of EGR transcription factors (der Krammer et al, JBC, 273:14538, 98). In various animal models for AD our M1 agonists improve mnemonic processes. Notably, AF150(S) restores cholinergic markers, working memory impairments (Fisher et al, J. Neurochem., 70:1991, 98) and mediates decreased tau hyperphosphorylation (Genis et al, J. Neurochem., in press) in apolipoprotein E-deficient mice.

Conclusions: M1 agonists may be useful both in a symptomatic treatment in AD (e.g. cognition, behavior), and as disease modifying agents: i) by compensating for possible dysfunctions of neurotrophines; ii) in reducing b-amyloids; iii) in impairing paired helical filaments formation. Due to the positive role of m1 mAChR on most of the identified "villains" or risk factors in AD, M1 agonists represent a unique, albeit not yet explored, therapeutic strategy in AD. Long term use of highly selective M1 agonists in early stage of AD patients and/or other populations at risk are needed to test this unifying concept.

Progress In Pharmacotherapy Of Alzheimer Disease: Symptomatic Stabilization With Cholinesterase Inhibitors
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The cholinergic system is the earliest neurotransmitter system of the brain to be affected in Alzheimer's disease (AD). During the last decade, a systematic effort to develop a treatment for AD has resulted into a series of new cholinergic drugs (cholinesterase inhibitors, muscarinic and nicotinic agonists). Among them, only cholinesterase inhibitors (ChEI) have been registered in USA and Europe, for such a specific indication. Clinical trials on several thousand patients in Japan, USA and Europe have confirmed the hypothesis that a steady state increase of acetylcholine in brain resulting from cholinesterase inhibition produces a significant cognitive improvement in mild to moderate AD patients. This clinical effect is long-lasting (12-24 months). Some ChEI have been proven to produce behavioral effects on AD patients (apathy, depression, agitation and hallucinations). The main effect of ChEIs on AD patients is to maintain cognitive function (measured with ADAS-cog) at a stable level over a 12 month period (stabilizing effect) as compared to the decline seen under placebo. This stabilizing effect raises questions such as: 1. whether or not the drug can slow down cognitive deterioration and 2. whether or not this effect is purely symptomatic or structural. Analysis and comparison of 6 ChEI clinical effects demonstrate a similar magnitude of improvement for aproximately the same time period. However, differences exist among various ChEI which can be explained with pharmacodynamic and pharmacokinetic properties. New in vitro and in vivo data point to cholinergic mechanisms of APP (amyloid precursor protein) regulation and ChE synthesis in brain involving three classes of cholinomimetic compounds (ChEI, muscarinic and nicotinic agonists) making them candidates for neuroprotective effects. Clinical verification of this hypothesis is in progress.

Over the last 10 years, considerable progress has been made through the application of molecular genetics to the study of the dementias. In particular, three genes have been shown to be important in the etiology of autosomal dominant Alzheimer's disease (AD), the APP, PS1 and PS2 genes. Mutations in all these genes have been shown to cause increases in the production of the peptide Ab42 in plasma of affected individuals as well as in transfected cells and transgenic animals: this peptide is found in the neuritic plaque: the pathognomic lesion of this disease. These genetic and molecular biological data form the underpinning of the amyloid cascade hypothesis. More recent data have shown that mutations in the tau gene lead to frontotemporal dementia to neurofibrillary tangle formation (NFT). As NFTs are the other pathological lesion in AD, this observation has profound implications for our understanding of this disease. Finally, mutations in the a-synuclein gene have been shown to be one (rare) cause of Parkinson's disease and to lead to Lewy body formation. Lewy bodies also occur in some cases of AD and both NFT and Lewy bodies occur in some cases of prion disease. Together, these findings point to pathogenic relationships between these different neurodegenerative diseases.

Transgenic Animal Models As Tools To Test Therapies For AD

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We have generated a transgenic mouse model that over-expresses mutant presenilin 1 (PS1) and the amyloid precursor protein (APP). The double transgenic mouse (PSAPP) develops amyloid deposits in the cingulate cortex at 10-12 weeks of age. With increasing age, the amyloid burden increases dramatically and spreads throughout the cortex and hippocampus. The composition of the deposits was examined using antibodies to different beta amyloid forms and was found to be very similar between mouse and human AD. Deposition is initially associated with reactive gliosis (GFAP immunoreactivity), but the response of astrocytes changes with increasing amyloid burden. There are also alterations in the cholinergic system in the frontal cortex suggesting that cholinergic cells in this region are particularly sensitive to amyloid deposition. The early deposition phenotype and low degree of variance between mice make these animals an ideal model system in which to screen beta amyloid modulating drugs. Secondary phenotypes such as cholinergic deficits that may be of relevance to the AD phenotype need to be explored further to establish how good a model of human AD the mice are.

Studies On Cortical Features Of Mice And Rat Transgenic Models

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Transgenic animal models allow the investigation of the impact of single or combined transgenes on Alzheimer's disease (AD)-like neuropathology. Cholinergic deficits are one of the most consistent landmarks of the disease. In collaboration with Dr. K. Duff of the Nathan Kline Institute (NY), we (T.P. Wong, T. Debeir and ACC) have examined transgenic mouse models (single transgenic lines: PS1M146L, and APP K670N,M671L, and a doubly transgenic line: APP K670N,M671L + PS1 M146L) that overexpress mutated AD-related genes [presenilin-1 (PS1) and the amyloid precursor protein (APP)] so as to investigate the effect of AD-related gene overexpression and/or amyloidosis on cholinergic parameters. At the time point studied (8 months), no apparent changes in either the size or density of cholinergic synapses were found in the PS1 M146L mutant relative to the nontransgenic controls. However, the APP K670N,M671L mutant showed a significant elevation in the density of cholinergic synapses in the frontal and parietal cortices. Most importantly, the double mutant (APP K670N,M671L + PS1 M146L), which had extensive amyloidosis, demonstrated a prominent diminution in the density of cholinergic synapses in the frontal cortex and a reduction in the size of these synapses in the frontal cortex and hippocampus. This study suggests a novel role of APP and a synergistic effect of APP and PS1 that correlates with amyloid load in the reorganization of the cholinergic network in the cerebral cortex and hippocampus at the time point studied (see Wong et al, J. Neurosci.19:2706,1999). We are presently extending these studies to the rat, as this species should have some experimental advantages. Our group, in collaboration with Drs L. Alhonen and J. Jänne at the Virtanen Institute (Kuopio), Dr. K. Duff of the Nathan Kline Institute (NY), and Drs. Baralle and Muro from the International Centre for Genetic Engineering and Biotechnology (Italy), is generating new transgenic rat models of AD pathology. The earliest generated line was coded UKUR25. This new rat transgenic line expresses human APP 751 with two mutations: the Swedish double mutation and the London mutation (FADx2), along with PS1 with the Finn mutation. Both transgenes are driven by the platelet-derived growth factor (PDGF) promoter and were injected in rat oocytes simultaneously by the standard pronuclear injection technique at the Virtanen Institute. Wistar rats (HsdBrl:WH) were used for transgenesis. Southern blot analysis showed that up to 30-40 copies of the transgene were present in the same lines and robust expression of the transgene was detected in the first set of tg rats using RT-PCR techniques

on cortical mRNAs. The presence of amyloidogenic APP fragments in the CNS of transgenic rats was detected using our own mouse monoclonal antibody (coded McSA1) and mAb 4G8 (Senetek PLC), which recognize epitopes 1-14 and 17-24, respectively of the Abeta fragment. This revealed, at the last analysis (6-11 month-old time points in the first tg, but not highest A-beta expressor, a marked accumulation of A-beta immunoreactive material inside neuronal cell bodies of the neocortex, dentate gyrus and hippocampus. Under electron microscopy this corrresponded to the presence of abnormal, large, distorted, multivesicular bodies in the soma of pyramidal neurons. In addition, A-beta immunoreactivity was found in axonal and dendritic processes as well as in glial extensions around cortical capillaries. Further functional and morphological characterization of this and other lines is underway.

Presenilins And Apoptosis: A Key Event In The Pathogenesis Cascade Of Alzheimer's Disease?

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The large majority of familial early-onset forms of Alzheimer's disease are linked to mutations in two genes recently identified, presenilins 1 and 2 (PS). PS mutations lead to an increase in the production of the long form of the amyloid peptide Ab42 possibly by physically interacting with the Ab precursor, APP, in the endoplasmic reticulum. In addition, a role for PS in modulation of apoptosis has been suggested by several reports.

We have analyzed in different cellular systems the modifications of apoptosis induced by expression of PSs. Transient transfection of PS2 in CHO fibroblasts leads to the induction of apoptosis as measured by Sytox staining (Molecular Probes). This induction can be detected as early as 24 h post-transfection. Both mutant (N141I) and wild type PS2 lead to apoptosis while PS1or other additional controls do not. Similar results were obtained with theTunel staining. In addition, we measured a marked decrease in reporter gene (SEAP, luciferase) expression upon PS2 co-transfection which could represent a convenient surrogate marker for the PS2-induced cellular stress/apoptosis. PS1 wt or mutant did not alter reporter gene expression and the PS2 effect could be reversed with a PS2 antisens construct. A C-terminal truncated PS2 extending only past the second transmembrane domain inhibited similarly reporter gene expression. The mutation on PS2 appears to accelerate the kinetics of this process.

To further confirm these findings in a more physiological

setting, we analyzed apoptosis in transgenics PS animals. A transgenic rat was generated expressing human PS1wt under the control of a modified HMG-CoA reductase promoter targetting transgene expression to neurons. The processing of hPS1wt into two characteristics fragments was documented as well as neuronal expression. Cultured primary cortical neurons derived from transgenic embryos display a greater level of spontaneous apoptosis at DIV7 than litter-mate non-transgenics. Upon trophic factor withdrawal, apoptosis was enhanced in transgenic cultures as analyzed with either DAPI or Tunel staining. Using a double-labelling technique, apoptosis in transgenic cultures was shown to be correlated to hPS1 expression.

These results further emphasize the role of PS1 in apoptotic cell death in the more physiological conditions of neuronal cultures. An increased sensitivity to PS1 expression in neurons as compared to CHO fibroblasts might likely explain the difference with transfected cells and will be further analyzed in PS2 transgenics. Involvement of a FAD gene in the regulation of apoptosis could suggest that apoptosis contributes to the neuronal loss in AD. Characterization of the apoptotic mechanisms in the PSs trangenic models should allow the identification of potential markers which could be analyzed in pathological AD samples to further test this hypothesis.

Tauopathies And Dementia

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The microtubule associated protein tau accumulates in abnormally hyperphosphorylated forms in Alzheimer's disease (AD) and in several other selected neurodegenerative disorders are referred to as tauopathies. These disorders are characterized by selective expression of different tau isoforms, their abnormal hyperphosphorylation, neuronal degeneration and dementia. Although abnormal hyperphosphorylation of tau is a common feature of this group of diseases, they differ from one another in involvement of selective tau isoforms, ultra structure of the tau aggregates/filaments and topography of these lesions. The phosphorylation of tau is not only determined by the activities of the protein kinases and protein phosphatases acting on this protein but also by its primary structure and conformational state. The AD abnormally hyperphosphorylated tau sequesters normal tau, resulting in disassembly of microtubules. The efficiency of this sequestration also varies from tau isoform to isoform. It is thus critical to understand the factors that lead to the expression, phosphorylation and sequestration of different tau isoforms.

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Glial Cells In Aging And Dementia

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In addition to beta-amyloid (bA4) aggregates and neurofibrillary tangles (NFT), glial response is a prominent pathological feature in Alzheimer's disease (AD). As the expression of mRNA of apolipoprotein E (ApoE), one of the riskfactors for AD, is detected in astrocytes and microglia is activated by amyloid precursor protein (APP) interest have been directed to the possibility to influence the progression of AD by influencing glial cell.

In a clinically and neuropathologically verified post-mortem material of 95 subjects significantly increased number of astrocytes (increase in the expression of glial fibrillary acidic protein (GFAP) and significantly increased number of activated microglia (upregulation of HLA DR expression) was noted. Furthermore a clinically and neuropathologically verified post-mortem material of 44 demented subjects without known non-steroidal anti-inflammatory medication showed significant increase in bA4 load and NFT counts with increased dosage of ApoE e4 allele. The number of GFAP expressing astrocytes and HLA DR expressing activated microglial cells also increased with the dosage of ApoE e4 but this change was not significant. The AD lesions such as (A4 load correlated significantly with astrogliosis and the counts of NFT's correlated significantly with the counts of activated microglia and both these correlation's were influenced by the ApoE genotype being abolished with addition of the ApoE e4 allele. Our results confirm that indeed the glial reaction is a phenomenon associated with the histopathological lesions of AD, and that this reaction is influenced by the ApoE genotype.

Epidemiological studies have indicated that non-steroidal anti-inflammatory drugs (NSAID) may have some therapeutic effect in AD and experimental studies have shown that microglia activation by bA4 is influenced by NSAIDs. We analysed 42 clinically and histopathologically verified demented patients fulfilling the histopathological CERAD criteria for definite AD, representing the end stage of brain degeneration. Half of the patients had received regular NSAID medication. Our results indicate that a regular NSAID use is associated with lower counts of astrocytes (significant) as well as with lower counts of activated microglia. The influence of NSAID was noted in all ApoE genotypes. Moreover in the ApoE e4/4 the influence was closest to be significant.

Based on our results one would expect a regular NSAID treatment to have a beneficial effect on the progression of the disease. The lack of significant differences for the activated microglia might however indicate an age or stage dependent

difference in glial response i.e activation rate. More age and stage related knowledge in glial response in humans is required in order to outline accurate pharmacological treatment strategies for AD patients carrying various riskfactors.

Complement Proteins As A Link Between A-beta Deposits And Inflammatory Response

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The current data support the view that in Alzheimer's disease (AD) brains the neuritic plaque is closely associated with a locally induced, non-immune mediated, chronic inflammatory response without any apparent influx of leucocytes from the blood. The concept that A-beta (Ab) peptide itself can induce a local inflammatory response received strong impetus by the finding that aggregated Ab peptide can bind and activate the classical complement pathway in an antibody independent fashion. Immunohistochemical studies have shown in AD brains the presence of the classical complement pathway proteins, complement regulatory proteins and several complement receptors especially in the vicinity of senile plaque. It will be discussed that the complement seems to be intimately involved in several crucial events in the pathological cascade as follows:
(i) It has been shown that C1q can strongly accelerate Ab fibrillogenesis.
(ii) Once fibrillar, the Ab-induced complement activation leads to the generation of C1q and of C4 and C3 fragments that decorate the neuritic plaques. In turn, these factors (especially the anaphylotoxins C3a, C5a) attract microglia that become activated and express the C3b receptors.
(iii) C3b receptors can potentially phagocytize the complement opsonized Ab fibrils and dystrophic neuronal elements in plaques. So, complement mediated mechanisms seem to be involved in both amyloid production and removal.
(iv) Finally, the formation of sublytic amounts of the complement membrane attack complex in neuritic plaques may iniate pathways that ultimately result in the death of neurons.

Recent work shows that cytokines associated with plaques (interleukin-1 and 6, tumour necrosis factor) stimulate human glial and neuronal cell cultures to secrete early complement proteins, but not C1-inhibitor. Our work, as well as that of others, indicates that the messenger levels of the early complement factors are increasingly expressed in AD brains, whereas C1-inhibitor is not. This could enable ongoing complement activation at sites of amyloid deposition, especially when C1-inhibitor is consumed and not replaced.

Besides inhibition of complement activation, C1-inhibitor is also involved in the regulation of coagulation, fibrinolysis and in the release of kinin. Consequently ischaemia-induced consumption of C1-inhibitor by these latter systems may allow an increased production of activated complement products, thereby facilitating the complement-mediated pathogenic mechanisms in AD.

The Relationship Between Neurodegeneration And Cell-Cycle-Related Events In AD

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Neurodegeneration in Alzheimer's disease (AD) is associated by the appearance of neuritic growth profiles that are aberrant with respect to their localization, morphological appearance, and composition of cytoskeletal elements, making a primary pathogenetic role of aberrancies of growth and proliferation regulating mechanisms in neurons very likely. During early stages of AD, a variety of growth factors and mitogenic compounds are elevated. Most of these factors mediate their cellular effects through activation of the p21ras -dependent mitogen activated protein kinase (MAPK) cascade, a pathway that is also involved in the regulation of expression and posttranslational modification of the amyloid precursor protein and tau protein. p21ras and other members of the MAPK cascade are highly expressed within tangle-bearing neurons and Ab-deposits. The small G-protein p21ras is a proto-oncogene which in transforming cells is involved in the regulation of the G0/G1 transition of the cell cycle mediated through co-operation with cyclin D1. Activation of the cyclin-dependent kinases (cdks) is negatively regulated by the interaction with proteins of the cyclin-dependent kinase inhibitor (cdki) family. Members of the INK4-family of cyclin dependent kinase inhibitors interacting with cdk4/6 such as p15INK4b, p16INK4a, p18INK4c and p19INK4d show an increased neuronal expression closely associated with tangle formation in AD suggesting an aborted attempt of neurons to re-enter the cell cycle. A high degree of structural neuronal plasticity in the adult brain, furthermore, predisposes neurones to tangle formation.

It is suggested that a process of neuronal dedifferentiation is a critical event in the pathomechanism of AD involving molecular events that are not compatible with the state of a neuron being irreversibly blocked from the re-entry into cell cycle, thus, ultimately leading to neuronal death.

The Role Of COX-2 In Alzheimer's Disease

Neurodegeneration

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Epidemiological studies indicate that non-steroidal anti-inflammatory drugs (NSAIDs) improve the clinical course of Alzheimer's disease (AD) (1,2). NSAIDs are potent inhibitors of cyclooxygenase (COX). We demonstrated that expression of COX-2 (but not COX-1) is induced by approximately 2-fold in Alzheimer's disease (AD) brain in comparison to age-matched controls (3). Further, COX-2 expression in both the normal and the AD brain is predominantly confined to neurons (3). These observations suggest that the clinical efficacy of NSAIDs may partially be derived from specific inhibition of neuronal COX-2 (4).

To further characterize the role of neuronal COX-2 in the development of AD, we generated transgenic mice with over-expression of human (h)COX-2 in neurons. Using primary neuron cultures derived from hCOX-2 transgenic mice we found that over-expression of hCOX-2 potentiated Ab(25-35)-mediated impairment of redox activity when compared to wild-type neuronal cultures (5). Further, we found that compared to wild-type littermates, hCOX-2 transgenic mice expressed higher levels of the complement component C1qB in the brain. Together, these findings indicate that neuronal COX-2 expression in AD may contribute to neurodegeneration by potentiating oxidative stress and/or by inducing a component of the complement cascade. Elucidating the function of neuronal COX-2 will clarify its role in the pathophysiology of AD.

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Regulation Of Cyclooxygenase-2 (COX-2) Expression In Brain Injury

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Background: Brain tissue has a widespread capacity to produce prostaglandins by COX-1 whose activity is dependent solely on availability of susbstrate, and by COX-2 which is induced by

inflammatory cytokines, strong depolarization and injury. Because prostaglandins as well as COX-generated superoxide play a role in neuronal injury, we studied the expression and regulation of COX-2 induction in ischemic brain damage.

Methods: An intraluminal model of transient focal ischemia, a 4-vessel model of global ischemia and a KCI-induced spreading depression (SD) model of migraine were used. The effect of various pre- and posttreatments with anti-inflammatory compounds and glutamate receptor antagonists on COX-2 expression was evaluated with quantitative in situ hybridization and immunocytochemistry.

Results: Both COX-2 mRNA and protein were induced in neurons after ischemia and spreading depression. The induction in the cortex was inhibited by an NMDA receptor antagonist MK-801 and inhibitors of phospholipase A2 (PLA2). In global ischemia MK--801 was inefficient and glucocorticoids inhibited COX-2 induction only in the cortex, whereas an AMPA receptor antagonist NBQX reduced hippocampal induction of COX-2. COX-2 expression and its inhibition was correlated with the extent of neuronal damage. Even though plasma glucose level strongly regulates several SD-induced immediate and late-response genes, hypoglycemia had no effect on COX-2 expression and hyperglycemia only slightly enhanced SD-induced COX-2 expression.

Conclusions: COX-2 induction is PLA2 and NMDA-receptor-dependent in cortical injury but AMPA-kainate-receptor-dependent in hippocampal ischemic damage. Plasma glucose modulate COX-2 expression slightly. Brain injury induces COX-2 expression exclusively in neurons suggesting that glial cells may not significantly contribute to prostaglandin production in vivo.

Cyclooxygenase In Alzheimer's Disease: Roles In Brain Inflammation And Neurodegeneration

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Epidemiological and clinical studies suggest that nonsteroidal anti-inflammatory drugs (NSAIDs) are beneficial in Alzheimer's, a disease characterized by neurodegeneration and prominent inflammatory changes. NSAIDs act by inhibiting cyclooxygenase, an enzyme occurring in constitutive (COX-1) and inducible (COX-2) isoforms. COX-2 plays a major role in peripheral inflammatory processes. Tissue culture studies show that astrocytes and microglia express COX-2 in response to pro inflammatory cytokines. Neurons also express cyclooxygenase and COX-2 inhibition is neuroprotective in some models of

neurodegeneration.

To address whether COX-2 plays a role in the CNS inflammatory response in vivo, we have quantified mRNA levels for 10 inflammation-related genes at early time points (4 and 24 h) in mice treated with a COX-2 selective inhibitor (NS-398) and subjected to single hemisphere brain irradiation. COX-2 expression was induced by injury. We also found that expression of many markers, including ICAM-1, IL-1b, TNFa, iNOS, MMP-9 and IL-6, was attenuated by NS-398, indicating that COX-2 dependent production of prostaglandins augments brain inflammatory responses. In contrast, mRNA levels for two chemokines, MCP-1 and MIP-2, were further induced by NS-398 following radiation injury. Thus COX-2 generated prostaglandins can also play a negative modulatory role.

In another set of studies we have examined COX-1 expression in human Alzheimer's and control brain. We find that CA3 and 4 hippocampal neurons make abundant COX-1 and expression levels are essentially equivalent in AD and control CA3 neurons. We also find that COX-1 immunoreactivity is readily detected in microglia regardless of activation state. However, numbers of COX-1 reactive microglia are increased in AD neocortex and these cells are associated with Ab plaques, suggesting that COX-1 may also contribute to AD pathogenesis.

These results support the use of NSAIDs in the treatment of brain inflammation and neurological diseases with an inflammatory component, such as Alzheimer's disease. Ongoing clinical trials with selective COX-2 inhibitors will help sort out the contribution of each isoform.

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Neurochemical Correlates Of Behavioural Disturbance In Prospectively-Assessed Patients With Dementia

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Although dementia is defined in terms of cognitive impairment, patients with Alzheimer's disease (AD) frequently suffer from behavioural and psychological symptoms including depression, delusions, hallucinations, aggression, wandering, sexual disinhibition and hyperphagia. These behaviours, some of which occur in almost all patients at some stage of the disease, cause significant problems to care-givers and often determine the need for institutionalisation. Increased understanding of the underlying biochemical mechanisms responsible is not only of considerable theoretical interest but may lead to the

development of treatments targeted at specific behavioural problems, and may provide the basis for prophylaxis.

For the most part these studies have relied on retrospective behavioural data, however the present study uses behavioural data collected prospectively using a standardised and evaluated method, the Present Behavioural Examination (PBE). Subsequently, other tests of behavioural disturbance have been developed, for example the neuropsychiatric inventory. The PBE interview was developed specifically for a prospective study of behaviour in 104 elderly patients with a clinical diagnosis of dementia who were living initially in the community with a carer. The PBE is a semi-structured interview administered every four months to the principal carer, which covers in detail the observable behaviour and psychological functioning of the subject over the previous four weeks. Four main syndromes have been identified: 1) aggression (physical and verbal), 2) overactivity (walking excessively, trailing and checking), 3) psychosis (hallucinations, delusions, anxiety) and 4) depression.

In the present study we have sought to determine whether the four syndromes identified in 35 pure AD cases during life have any neurochemical correlates. We have examined markers of the cholinergic, dopaminergic and serotonergic systems together with markers of synapses.

Significant correlations between neurochemical measures and prospectively assessed behaviour

	Aggression	Overactivity	Psychosis	Depression
5-HT terminals				Decreased
5-HT2A receptors			Increased	
ChAT activity	Decreased			
Dopamine D1	Decreased	Decreased		Increased
Muscarinic M2			Increased	

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AD-Related Proteins And Synaptic Markers In Rats With Entorhinal Cortex Lesions

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The cause of Alzheimer's Disease (AD) remains unknown, but there is a consensus regarding the spread of pathology. The entorhinal cortex is the area considered to be the first affected, with the subsequent involvement of the hippocampus. Glutamatergic neurones from the entorhinal cortex and cholinergic neurons from the medial septum form the major excitatory inputs to the hippocampus. Both of these neuronal systems are considered to be affected early in Alzheimer s disease and their loss significantly correlates with the degree of cognitive impairment.

Our hypothesis is that a failure of neurotransmission in AD may contribute to the spread of pathology. We predict that partial deafferentation lesions of the hippocampus in rats would induce changes in the expression of AD-related proteins. We have studied the effects of removing the glutamatergic input to the hippocampus on the regional distribution of AD-related proteins, the amyloid precursor protein (APP) and tau, and synaptic markers, such as the vesicular-associated protein synaptophysin and the presynaptic membrane proteins SNAP-25 and syntaxin. A secondary aim of the project was to investigate the possible association of a certain synaptic protein with neurons using particular neurotransmitters.

Unilateral eletrolytic lesions of the entorhinal cortex were assessed by visual examination of sections after Nissl staining, acetylcholinestarase histochemistry and reactive gliosis. An immunohistochemical method was used to study the expression of the proteins. APP mRNA levels were detected by in situ hybridization using a radiolabelled riboprobe containing the b-amyloid sequence. Changes in immunoreactivity after lesioning were quantified by image analysis system and special care was taken to minimize the possible sources of variability. Results were analyzed by a multivariate ANOVA.

Following APP immunostaining with the antibody 22C11, pyramidal cells were strongly labelled, with the highest levels in the CA3 field, followed by hilus>CA1>molecular layer>granule cells of the dentate gyrus. This pattern of expression correlates with the APP mRNA levels found using in situ hybridization. At 3 and 7 days after entorhinal cortex lesion, no changes in immunostaining were found in any of the areas studied. However, 1 month after the lesion, significant increases in APP immunostaining were noticeable in the CA1 and CA3 regions and in the granule cell layer. These increases were preceded by increases in APP mRNA in the same regions and observed 7 days after entorhinal cortex lesion. No changes in tau protein expression have been found at any of the time points using different antibodies (MC-1, ALZ-50, TG-3). 7 days after entorhinal cortex lesion, a significant decrease in

SNAP-25 immunostaining was observed in the perforant path terminal zone (OML), and the image analysis indicate this decrease to be about 60%. No changes were seen in synaptophysin and syntaxin immunostaining after ECL. These results were compared with those obtained after lesioning, using 192-IgG-saporin, the cholinergic projections arising from the medial septal area. After the cholinergic lesion, increases in APP immunoreactivy were detected in several regions (CA1, CA3, granule cell layer and hilus) 3 and 7 days after the lesion, but by 1 month none of these increases reached statistical significance. APP mRNA levels were decreased in most of the areas studied. Decreases in SNAP-25 were also observed in the hilus 7 days after the lesion.

The present results imply that loss of excitatory inervation to the hippocampus leads to increases in APP immunostaining within neurons. This could be related to the purported role of APP in the promotion of neuronal survival and protection of neurons against insults. But also the reduction of excitatory neurotransmission accompanying these lesions may lead to an increase in the amyloidogenic metabolism of the increased concentration of APP and to b-amyloid deposition in vulnerable species. This study provides no clear evidence of selective enrichment of individual synaptic proteins in neurotransmitter specific synapses. However, SNAP-25 could be the most sensitive marker to detect early synaptic losses.

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with hormone activity. These studies demonstrated that the events triggered by ER activation in this cell line recapitulate what is actually occurring "in vivo" in neural cells (1-3). We therefore proposed the SK-ER3 cell line as a suitable system for the analysis of ER molecular activity in neural cells.

More recently, by ddPCR technology we were able to identify a panel of genes under estrogen control (4). Among these, the gene NIP-2 generated a certain interest since its protein product is known to interact with the anti-apoptotic protein Bcl-2. Interestingly, we demonstrated that Nip-2 mRNA levels are negatively regulated by estrogens in SK-ER3 cells. This finding led us to postulate a role for estrogens in the mechanism of SK-ER3 cells apoptosis. Indeed, we were able to prove that estrogens significantly protect cells from the death process induced by glucose deprivation or other toxic insults. The anti-apoptotic effect of estrogens appears to be mediated by ER activation, since it is observed at low estradiol concentrations, can be blocked by the presence of ER antagonists and it does not occur in neuroblastoma cells not expressing ER. Studies are in progress to determine the levels of Nip-2 expression in rat hippocampus after apoptotic and hormonal stimuli, as this specific brain area represents the primary target for apoptosis in AD patients.

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Effect Of Estrogen On Neuronal Plasticity

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It has been demonstrated by many studies in rats that, following entorhinal cortex ablations, the dentate gyrus shows an early degeneration of the lesioned axons and terminals, followed later by a sprouting response of non-lesioned axons. We hypothesized that this response would be altered in female mice receiving estrogen replacement therapy (ERT). Therefore we lesioned the entorhinal cortex in these and in control animals, and analyzed the response following the lesions. Mice were ovariectomized, received ERT or not, and the entorhinal cortex was unilaterally lesioned by injections of ibotenic acid; four weeks later the animals were sacrificed and transcardially perfused. The brains were cut and stained for markers that analyze sprouting, the most consistent changes were present in the material stained for synaptophysin, a protein that marks

presynaptic terminals.

Following unilateral lesions of the entorhinal cortex of the mouse the ipsilateral hippocampus demonstrates sprouting. The increase in expression of synaptophysin was present in the outer molecular layer of the dentate gyrus and in stratum lacunosum moleculare of area CAI of the hippocampus. Both the control mice and the mice receiving low dose and high dose ERT display a normal sprouting response to the entorhinal cortex lesion. However, in mice that were ovariectomized and did not receive ERT, the sprouting response was substantially reduced. ERT treatment in these ovariectomized mice did not change the response of the brain to lesions compared to non-ovariectomized mice, but the lack of estrogen leads to a reduction in the sprouting response in the hippocampus following entorhinal cortex lesions.

precursor protein (APP) itself, by finding that IL-1 also upregulates the production of APP in astrocytes, but at the translational rather that the transcriptional level. These results suggest potential specific targets for anti-inflammatory therapy for AD that will go beyond the promising effects of the general anti-inflammatory drugs tested thus far.

Complement Driven Inflammatory Mechanisms In Alzheimer's Disease

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There is accumulating evidence linking the neurodegeneration that occurs in Alzheimer's disease (AD) with a local inflammatory. The source of this inflammatory response is hypothesized to derive from a chronic, low level activation of the complement pathway (C'). Elements of the classical complement pathway (CC') have been co-localized with fibrillar plaque deposits of the beta-amyloid peptide. A direct linkage between beta-amyloid and C activation was fisrt established by Rogers et al. (1992), who showed that beta-amyloid could activate the C1 complex of the CCP in an antibody independent manner. Jiang et al. (1994) then demonstrated that C1 activation was dependent upon aggregated forms of the C1q A-chain. C1 activation by beta-amyloid is dependent of the peptide being in a beta-pleated sheet, multimeric form. Moreover, at least two negatively charged residues, asp 7 and glu 11, appear to be critical for the activation of human C1 by beta-amyloid. More recently, some of the pathological features of AD have been replicated in mouse transgenic models, including the deposition of beta-amyloid into senile plaque-like deposits and co-localization of activated astrocytes and microglia to these structures. However, in the transgenic models studied thus far, neuronal loss and deficits in cognitive ability have not been generally observed.

We have previously demonstrated that a specific sequence of the human C1q A-chain is critical for binding to beta-amyloid and inducing the activation of complement. The sequence of this region of mouse C1q differs significantly from the human in that two of three arginines capable of interacting with fibrillar forms of beta-amyloid are missing in the mouse A-chain sequence. A peptide based on the mouse C1q A-chain sequence was synthesized and found to be ineffective at blocking the Abeta-induced activation of human complement, in contrast to the inhibitory effect seen with the human C1q A-chain peptide. Direct comparison of mouse and human serum showed that human complement was activated more effectively by A-beta. There is accumulating evidence linking the neurodegeneration that occurs in Alzheimer's disease AD with

a local inflammatory. The source of this inflammatory response is hypothesized to derive from a chronic, low level activation of the complement pathway (C'). than by mouse complement. In contrast, mouse complement was activated more effectively by both human and mouse IgG than was human complement. Thus, the significantly lower level of activation of mouse complement by beta-amyloid as compared to human complement may contribute to a less robust amyloid driven cascade of complement activation, inflammatory events and neurodegeneration in transgenic models even though they do develop extensive AD-like plaque pathology.

With regard to the development of transgenic models of AD that faithfully replicate all aspects of the disease, it will be important to fully understand the relative contribution of the various pathological markers that have been linked to the disease. If the activation of the classical complement pathway by beta-amyloid does contribute significantly to the pathogenesis of AD then a further humanization of the mouse genome may be necessary to effectively trigger the same level of A-beta-driven complement activation and inflammation that occur in AD.

neuronal maintenance and repair mechanisms. In the present study we examined the hypothesis that apoE plays an important role in neuronal maintenance and repair and investigated the cellular and molecular mechanisms underlying the isoform specificity of these effects.

Behavioral and neuronal assessment of apoE-deficient (knockout) mice revealed specific memory impairments in these mice which are associated with presynaptic derangements in brain projecting neurons whose rank order is similar to that observed in AD (cholinergic>adrenergic>serotonergic> dopaminergic). Closed head injury exper-iments revealed that the apoE-deficient mice are particularly susceptible to head injury and exhibit an impaired ability to recover from this trauma. The possibility that the observed effects of apoE on neuronal maintenance and repair are related to distinct interactions between apoE, the neuronal cytoskeleton and membrane neogenesis was investigated by the following experiments.

Measurements of the levels of phosphcrylation of the cytoskeletal protein tau of apoE-deficient mice revealed that it contains a hyperphosphorylated "hot spot" which is localized N-terminally to the micro tubule binding domain of tau. Prolonged cholinomimetic treatment with an M, agonist (AF150(S)) reversed the cognitive and neurochemical deficiencies of the apoE-deficient mice and markedly diminished the extent of their tau hyperphosphorylation. These animal model findings are consistent with the assertion that some of the neuropathological effects of apoE4 in AD may be due to loss of a protective function which results in excess tau phosphorylation, and the consequent destabilization of the neuron Al cytoskeleton.

The possibility that apoE also exerts its neuronal function by affecting membrane neogenesis was examined by in vitro tissue culture experiments in which the effects of exogeneously added apoE4 and apoE3 on phospholipid synthesis were examined. This revealed that apoE3 and apoE4, when added by themselves, had no effect on phospholipid synthesis. However, when apoE3 (10 (g/ml) was added to the cultures in the presence of an exogenous lipid (e.g (VLDL) it stimulated the synthesis of phosphatidylcholine. By contrast, apoE4 under similar conditions slightly inhibited this process. These tissue culture findings suggest that the neuropathological effects of apoE4 on neuronal function may be mediated by isoform-specific effects on neurite outgrowth and synaptic plasticity.

The relative pathophysiological importance of the effects of apoE on the neuronal cytoskeleton and on neurite outgrowth and their interplay with other processes such as the inflammatory system will be discussed.