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The Third Kuopio Alzheimer Symposium

Microteknia, Kuopio, Finland
March 21-22, 2003

Program
and
Abstracts

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Dear Colleagues and Friends,

You are cordially invited to participate into the Third Kuopio Alzheimer Symposium, which will be held in Kuopio, Finland, March 21-22, 2003.

This series of meetings will focus on different important areas of Alzheimer's disease research. Our goal is to bring experts together and provide a forum for new ideas for future research. The First meeting was held 28-30 January 1999. Although the outside temperature was freezing (-30°C), the atmosphere in the meeting was warm and exited. The second meeting was held in March 2000 in order to provide the participants with a little bit milder climate and more day light. We succeeded in composing interesting programmes, which were appreciated by about 250 participants in each meeting.

The programme in the forthcoming third meeting will focus on exciting new data concerning vascular risk factors and Alzheimer's disease. The role of cholesterol in the pathogenesis, treatment and prevention will be reviewed. We will also hear whether there are data showing that by treatment of hypertension we can prevent Alzheimer's disease. We will also have overviews on other dementias such tauopathies and dementia with Lewy bodies. How close we are to the solution of therapy of Alzheimer's disease, will be covered in the therapy session. We will hear about the current status of cholinergic and gutaminergic treatment approaches as well as a critical review on anti-amyloid therapy. How to assess disease modifying efficacy of pharmacological agents in Alzheimer's disease, will also be discussed.

I warmly welcome all to Kuopio to enjoy this exciting scientific meeting, which will also provide you an opportunity to experience the Finnish spring-winter.

Hilkka Soininen
Professor
Chairperson of the Organizing Committee

The Third Kuopio Alzheimer Symposium

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Oral Program

Friday, March 21, 2003

Opening ceremonies

09.00 Vice Rector Katri Vehviläinen-Julkunen
University of Kuopio

09.05 Professor Hilkka Soininen
Department of Neuroscience and Neurology, University of Kuopio

Plenary lecture

09.15 **Developing a Diagnostic test for Alzheimer's Disease**
Kaj Blennow, Sweden

09.45 **Coffee Break**

Main Theme Cholesterol and Dementias

Sponsored by Pfizer

Chairpersons: Tobias Hartmann and Pentti Tienari

10.15 **Cholesterol and The Brain**
Dieter Luthjohann, Germany

10.45 **Cholesterol and APP Metabolism**
Tobias Hartmann, Germany

11.15 **Cholesterol as Risk Factor for AD**
Mia Kivipelto, Sweden

11.45 **Special lecture**
Treatment Response with Donepezil Diifferentiates Vascular Dementia from Alzheimer's Disease
David Wilkinson, USA

12.15 **Lunch Break**

DEVELOPING DIAGNOSTIC TESTS FOR ALZHEIMER'S DISEASE

Kaj Blennow, MD, PhD, Professor

The Sahlgrenska Academy at Göteborg University
Institute of Clinical Neuroscience, Experimental Neuroscience Section

The possibility to provide Alzheimer's disease (AD) patients symptomatic treatment with AChE inhibitors has made patients seek medical advice very early in the course of the disease, when symptoms are vague and difficult to distinguish from memory problems associated with normal aging. This has created a great need for biochemical diagnostic markers of AD. Forthcoming disease-arresting drugs (e.g. β/γ-secretase inhibitors) will make this need even larger.

During the last 10 years, research efforts to develop biochemical diagnostic tests for AD have resulted in specific ELISA methods for the three central pathogenic processes in AD, including axonal and neuronal degeneration (total tau, T-tau), β-amyloid mismetabolism and deposition (Aβ42), and the phosphorylation state of tau and development of tangles (P-tau).

These CSF biomarkers have now been evaluated in numerous papers. All studies have consistently found a marked increase in CSF T-tau and a decrease in CSF Aβ42 in AD, with sensitivity figures ≈90%. The specificity is also high against normal aging, depression, and Parkinson's disease, but lower against other dementias. The addition of P-tau have been found to increase the specificity, since increased levels are not found in other dementias (e.g. frontotemporal dementia).

Importantly, these CSF-markers are positive also in patients with mild cognitive impairment (MCI), and recent studies have shown that CSF markers may help to differentiate MCI cases that will progress to AD with dementia from those who have stable MCI.

We use CSF T-tau, P-tau and Aβ42 in clinical routine in our laboratory. CSF samples are sent for diagnostic purposes from clinicians all over Sweden and ELISAs are run each week as clinical neurochemical routine analyses. Also in this setting, the analytical variation is within the range expected for immunoassays, and we find high sensitivity and specificity figures also in an unselected community-based patients.

CHOLESTEROL AND THE BRAIN
24S-HYDROXYCHOLESTEROL: A MARKER OF BRAIN CHOLESTEROL METABOLISM

Dieter Lütjohann

Department of Clinical Pharmacology, University of Bonn

The enzymatic conversion of CNS cholesterol to 24S-hydroxycholesterol, which readily crosses the blood-brain-barrier, is the major pathway for the elimination of brain cholesterol and the maintenance of brain cholesterol homeostasis. The enzyme mediating this conversion has been characterized at the molecular level (CYP46), but the mechanisms of its regulation are not yet known. Like other oxysterols, 24S-hydroxycholesterol is efficiently converted into normal bile acids or excreted in bile as its sulphated and glucuronidated form. The levels of 24S-hydroxycholesterol in the circulation are decreasing with age in infants and children. In adults, however, the levels appear to be stable.

There is accumulating evidence pointing toward a potentially important link between cholesterol, β-amyloid, and Alzheimer disease. Plasma concentrations of 24S-hydroxycholesterol from Alzheimer and vascular demented patients are significantly higher compared with healthy subjects. Variations in genetic background, time of disease onset, and severity of dementia are potential sources of variance. Measurement of 24S-hydroxycholesterol levels in the cerebrospinal fluid may provide more accurate data describing the progression of neurodegeneration.

Inhibitors of cholesterol biosynthesis, also termed statins, seem to have a reductive influence on the generation of the amyloid precursor protein, the neuronal secretion of β-amyloid, and on cholesterol de novo synthesis. Recent epidemiological studies indicate that the prevalence of diagnosed AD and vascular dementia is reduced among people taking statins for a longer period of time. High-dose simvastatin treatment (80 mg/day) in patients with hypercholesterolemia leads to a significant decrease of serum concentrations of brain-specific 24S-hydroxycholesterol and indicates a diminished cholesterol metabolism in the brain. Treatment with high-dose simvastatin in normocholesterolemic Alzheimer patients at early stages of the disease for 26 weeks results in a significant decrease of Aβ-levels in cerebrospinal fluid. This decrease correlates with the reduction of 24S-hydroxycholesterol.

We conclude that high-dose simvastatin treatment in early stages of Alzheimer disease may result in delay of the pathogenesis of β-amyloid, reasoned by a lowering of brain cholesterol metabolism.

CHOLESTEROL AND AMYLOID PRECURSOR PROTEIN METABOLISM

Tobias Hartmann

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Research from various fields of Alzheimer's disease (AD) research identified in the very recent years a role for cholesterol in AD. Retrospective epidemiological studies show dramatic difference in AD statin users and nonusers. *In vivo,* cholesterol feeding increased plaque formation and inhibition of cholesterol synthesis reduced cerebral Aβ40 and Aβ42 production. Clinical studies verify this concept because statins reduced Aβ blood levels and very recently we could show that Simvastatin at 80mg/day reduces cerebrospinal fluid Aβ levels significantly after 6 month of treatment.

From a cell biological point of view these results identify a new and completely unexpected link between cholesterol, cholesterol trafficking and the regulation of enzymatic activity central to AD. On the cellular level Aβ production depends on the enzymatic cleavage by APP-secretases. When deciphering the molecular mechanisms involved, we found that sub-cellular cellular lipid composition and lipid trafficking regulate the balance between the amyloidogenic and the nonamyloidogenic pathway. Lipid targeted treatments revealed a complex lipid dependent regulation of APP secretases in neurons. *(1)* β-secretase activity is reduced upon cholesterol depletion, as is γ-secretase activity. Remarkably, these secretases hardly share any structural or other homologies; still the same cholesterol lowering treatments inactivates them. Moreover, these effects are additive to each other, thus a small inactivation of both enzymes results in a pronounced loss of Aβ, as revealed by cholesterol manipulations restricted to different sub-cellular compartments. *(2)* This raises the possibility that cellular cholesterol trafficking is involved in Aβ generation. LDL derived cholesterol uptake is critically dependent on the function of the NPC1 protein localized to a late endosomal compartment. Exposure of neuronal cells to cholesterol transport / NPC1 function -inhibiting agents (Imipramine or U18666A) resulted in decreased α-secretase activity. In contrast, ?-secretase activity was enhanced, increasing the production of Aβ40 and Aβ42. These experiments illustrate the independent and mechanistically separate nature cholesterol treatments have on the different constituents of the Aβ generating molecular machinery. Blocking the intracellular cholesterol trafficking at the level of late endosomes also resulted in a parallel increase of cholesterol, presenilin (PS) and Aβ42 in specific vesicles relevant for cholesterol trafficking. The unexpected colocalization of certain ER markers indicates that block of cholesterol trafficking prevented export of APP-CTFs and PS from these vesicles. Moreover, it indicates that under these conditions ?-secretase is active in a compartment previously thought to play a role only in cholesterol trafficking. PS is mainly localized distant from sites of secretase activity. Transient fusion of these vesicles may help to understand this

spatial paradox. Taken together these data provide a rational for cellular mechanism explaining the epidemiological and prospective clinical data suggesting a beneficial role of low cholesterol levels in AD or dementia in general. Furthermore, our data provide evidence that reduced Aβ production may not be achieved by reducing the mean cholesterol content of neurons, but rather by alterations in cellular cholesterol distribution, uptake and storage.

How is it possible that a complex protein processing machinery, as it is found with APP and its secretases, is so intimately linked with the cellular lipid physiology? The best-known example for this is the SREBP/SCAP complex that regulates cholesterol and other lipids homeostasis. In fact there are multiple striking analogies between both protein-processing pathways. Obviously, the protease-lipid link makes perfect sense for SREBP and cholesterol regulation. However, it remains currently very difficult to explain why lipids would control AD protein processing and activity, as they have never been envisioned to play an active part in lipid homeostasis. Nevertheless, cholesterol is the only known natural and non-genetic factor to regulate A? generation and recently several new findings seem to tighten this link further towards a more active role of APP and other AD proteins in cholesterol homeostasis. Membrane diameter and composition appear to dictate the important cleavage site determination, affecting the AD relevant Aβ40/Aβ42 ratio and LRP, and important regulator of lipid homeostasis, has been identified as substrate of the APP processing complex.

With this knowledge at hand AD lipid research is facing two major frontiers. To provide enhanced treatment approaches and to better understand the biological design that makes APP a lipid target.

CHOLESTEROL AS RISK FACTOR FOR ALZHEIMER'S DISEASE

Miia Kivipelto

Karolinska Institutet, NEUROTEC, Division of Geriatric Medicine and Geriatric Epidemiology, Huddinge University Hospital, Stockholm, Sweden

Hypercholesterolemia is a known risk factor for atherosclerosis and coronary artery disease, both of which have been associated with AD. The apolipoprotein E ε4 allele, the most important genetic risk factor for AD, is associated with elevated serum cholesterol. High dietary intake of fat and cholesterol has shown to be related with an increased risk of dementia and AD. Animals fed with cholesterol rapidly accumulate β-amyloid in the brain. Experimental research has revealed that high cholesterol levels may directly induce AD neuropathology by modulating APP metabolism, and that depletion of intraneuronal cholesterol inhibits β-amyloid production *in vitro* and *in vivo*. Two independent, prospective population-based studies have reported an association between elevated serum cholesterol levels at midlife and AD later in life. Cholesterol values often fall before the manifestation of dementia, which may explain at least partly the inconsistent/negative results from some cross-sectional or short-term follow-up studies on this issue. Three recently published clinical studies have reported significantly reduced rates (up to 70%) of dementia and AD in subjects who had used statins as cholesterol reducing drugs. However, two recent randomized studies (HPS and PROSPER) found no significant effect of statin treatment on cognitive functions. Both of these studies had relatively short follow-up times (3-5 years) regarding to the development of dementia. Besides in the prevention, lipid-lowering drugs might have a role also in the treatment of AD. A recent, relatively small (n = 44), 26-week randomized, placebo-controlled study reported that simvastatin decreased the β-amyloid levels in the cerebrospinal fluid and slowed the progression of disease in normocholesterolemic patients with mild AD. These observations may have valuable implications for future strategies of prevention and treatment of AD.

DONEPEZIL PROVIDES COGNITIVE AND GLOBAL BENEFITS IN PATIENTS WITH VASCULAR DEMENTIA

DG Wilkinson,

Memory Assessment and Research Centre, Moorgreen Hospital, Southampton, UK

Background: As evidence suggests that vascular dementia (VaD) is associated with a cholinergic deficit, patients with VaD may benefit from cholinesterase inhibitor therapy.

Objective: Evaluation of the efficacy and tolerability of donepezil in patients with probable or possible VaD.

Design: A randomized, double-blind, placebo-controlled, 24-week study (Study 308).

Methods: A diagnosis of probable or possible VaD according to NINDS-AIREN criteria was required for inclusion; patients with a prior diagnosis of Alzheimer's disease were excluded. Patients were randomized to receive placebo, donepezil 5 mg/day or donepezil 10 mg/day (5 mg/day for first 28 days). Results are reported for intent-to-treat observed cases.

Results: 616 patients were enrolled (193 placebo, 208 donepezil 5 mg/day, 215 donepezil 10 mg/day); 76% had probable VaD and 24% had possible VaD. Both donepezil-treated groups showed significant improvements in cognition compared with placebo (ADAS-cog mean change from baseline score effect size at Week 24: donepezil 5 mg/day, -1.60, P=0.006; donepezil 10 mg/day, -2.12, P<0.001). Greater improvements in global function were observed with both donepezil groups than with the placebo group (% patients showing improvement on the CIBIC-plus at Week 24: placebo, 26%; donepezil 5 mg/day, 44%, P=0.006; donepezil 10 mg/day, 35%, P=0.08; overall treatment P=0.011). Donepezil was well tolerated in this population, with low withdrawal rates due to adverse events (placebo, 8.8%; donepezil 5 mg, 10.1%; donepezil 10 mg, 16.3%).

Conclusions: Donepezil is an efficacious and well-tolerated treatment for patients with probable or possible VaD, and may have an important role in the management of these patients.

Friday, March 21, 2003

Main Theme **Vascular Mechanisms in Alzheimer's Disease**

Sponsored by Janssen-Cilag

Chairpersons: Laura Fratiglioni and Timo Erkinjuntti

13.00 **Vascular Risk Factors in Alzheimer's Disease**
Charles DeCarli, USA

13.30 **Blood Pressure and Dementia**
Francoise Forette, France

14.00 **Contribution of Vascular Lesions To AD Pathogenesis**
Ray Kalaria, UK

14.30 **Coffee Break**

15.00 **Does Treatment of Hypertension Prevent Dementia?**
Laura Fratiglioni, Sweden

15.30 **Special Lecture**
Galantamine in Probable VaD and AD with Cerebrovascular Disease – Influencing Outcomes Across the Domains
Timo Erkinjuntti, Finland

Evening Programme

19.00 **Posters and Get-Together Party**

VASCULAR RISK FACTORS IN ALZHEIMER'S DISEASE

Charles DeCarli, MD

Associate Director, Alzheimer's Disease Center
University of California at Davis

Dementia is common to the elderly, particularly to those over age. While Alzheimer's disease (AD) is the most common cause for dementia amongst these individuals, cerebrovascular disease (CVD) is also quite common to the elderly. It is, therefore, not surprising that concurrent CVD is often seen in older dementia patients even though they may have a slowly progressive dementing illness most consistent with AD. Unfortunately, the impact of CVD or Cerebrovascular Risk Factors (CVRFs) on AD is not well understood, and the presence of concurrent CVD often causes diagnostic confusion for the treating physician, potentially limiting effective care for these individuals. With the advent of better methods to detect CVD, the interaction between CVD and neurodegenerative dementias, such as AD are now being explored and clarified. During my brief presentation, I will examine the impact of CVD on cognitive function during normal aging as well as in the setting of mild cognitive impairment (MCI) and dementia. Since my primary purpose will be to examine the interaction between AD and CVD, I will focus on studies of CVD caused by athero- or arteriosclerotic processes and limit discussion to studies that examine the impact of asymptomatic CVD on cognition where the role of CVD in the dementia process is less well understood.

BLOOD PRESSURE AND DEMENTIA

Françoise Forette

Hôpital Broca, CHU Cochin, University Paris V

The necessary requirements to consider that a risk factor is a good candidate for dementia prevention are the following: A statistical association between the exposure to the factor and the risk of dementia must be present without bias. The exposure to the factor occurred before the onset of the disease. The association must be plausible and coherent with the natural history and biology of the disease. The preventive effect of the risk factor modification must be replicated by at least one other study. High blood pressure fills these requirement.

Most longitudinal studies show a correlation between high blood pressure levels and the occurrence of cognitive impairment, of Vascular Dementia and often but not always Alzheimer's disease 15 to 20 years later. (Framingham study, Honolulu Asia Study, Eva Study, Gothenborg study...).

Hypertension increases the risk for multiple, small, large, bilateral strokes, for ischemic white-matter lesions (hyalinosis and narrowing of small perforating arteries) and for degenerative lesions (fibrillary tangles)..Vascular causes of cognitive impairment and Alzheimer's disease process coexist and interact (amyloid angiopathy) and cerebrovascular lesions decrease the threshold of dementia in individuals with AD lesions as shown in post-stroke dementia.

Two randomized, placebo-controlled trials using blood-pressure lowering agents, the SYST-EUR and PROGRESS demonstrated a reduction in the incidence of dementia. Further prospective dementia prevention trials comparing different classes of drugs are urgently needed to better determine the mechanism of dementia prevention study.

JF Dartigues et al. Epidemiology of dementia. Protective factors. In Alzheimer's Disease and related Disorders Annual. Ed. Gauthier S Cummings J. Pub; Martin Dunitz

CONTRIBUTION OF VASCULAR LESIONS TO AD PATHOGENESIS

Raj Kalaria

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Cerebrovascular changes are not exclusive to vascular dementia (VaD) or multi-infarct dementia. Our previous observations in a large autopsy series of cases have shown profound brain microvascular lesions in Alzheimer's disease (AD). It is not unlikely that the microvascular changes are compounded by the presence of cerebral amyloid angiopathy (CAA), a frequent lesion in AD. However, AD pathology was also associated with microinfarcts and to lesser extent large infarcts and CAA-related intracerebral haemorrhages. Other features include periventricular and deep white-matter changes or leukoariaosis seen upon magnetic resonance imaging and at autopsy in more than 40% of the late-onset AD cases. Such brain vascular pathology in AD may arise from systemic vascular or cardiovascular disease. Alternatively, VaD patients bear AD-type of pathology at autopsy and these also reveal neurochemical abnormalities consisting of deficits in presynaptic cholinergic indices related to the basal forebrain neurones. We also noted Abeta deposits and tangles were present in 43% of VaD patients with a small volume (<15 ml) of macro-infarction. These findings corroborate the importance of microvascular disease rather than macroscopic infarction as the critical substrate in VaD and also implicate cholinergic deficits in VaD. Is it possible that the microvascular lesions or multiple mircoinfarcts are the underlying primary trigger for subsequent Alzheimer pathology in late-onset AD? Evidence to implicate neovascularisation in AD pathogenesis will also be reveiwed. Treatment strategies that improve the dynamics of the cerebral circulation and chronic hypoperfusion would be rational targets for dementia.

Supported by the MRC, Alzheimer's Research Trust (UK) and Alzheimer's Association (USA).

DOES TREATMENT OF HYPERTENSION PREVENT DEMENTIA?

Laura Fratiglioni

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Numerous population-based studies have indicated that long-term hypertension is a risk factor for dementia and cognitive impairment. It is therefore biologically plausible to hypothesize that active treatment of hypertension may prevent dementia and preserve cognition. Evidence from randomised clinical trials and population-based studies, however, is still limited and controversial.

Randomised clinical trials. The SHEP trial did not show any benefits of diuretic and beta-blocker treatment on cognition and dementia. Treating moderate hypertension in older adults had no influence on subsequent cognitive function in the Medical Research Council's treatment trial of hypertension. Conversely, the Syst-Eur trial initially showed that in old people with isolated systolic hypertension the active treatment of hypertension with nitrendipine could decrease dementia incidence by 50% over 2 years. This finding was confirmed by the extended follow-up.

Observational studies. A few population studies have shown that antihypertensive medication is related to a reduced risk of dementia and cognitive impairment. In the Kungsholmen Project, antihypertensive drug use at baseline could reduce dementia and Alzheimer's disease risk by 20~30% over a 6-year period. Both high systolic and low diastolic pressure were associated with an increased risk of dementia, but the association between low diastolic pressure and high risk of dementia was evident only among individuals who used antihypertensive drugs.

Antihypertensive treatment in at risk populations. Antihypertensive drug use could diminish the risk-effect of *APOE* e4 on Alzheimer's disease, and modify the combined effect of blood pressure and *APOE* e4 on the development of the disease.

In summary, antihypertensive treatment appears to reduce the risk of dementia and Alzheimer's disease among old people with high blood pressure. The effect modification of antihypertensive drug use on the genetic linkage between *APOE* e4 and dementia needs to be further confirmation.

GALANTAMINE IN PROBABLE VASCULAR DEMENTIA AND ALZHEIMER'S DISEASE WITH CEREBROVASCULAR DISEASE – INFLUENCING OUTCOMESS ACROSS THE DOMAINS

Timo Erkinjuntti
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Sean Lilienfeld
Janssen Research Foundation, Titusville, New Jersey, USA

BACKGROUND: Galantamine, a novel therapy with a dual mode of action, inhibits acetylcholinesterase and modulates nicotinic receptors. It has shown sustained effects on cognition, global function, activities of daily living (ADL) and behavior in patients with mild-to-moderate Alzheimer's disease (AD).

OBJECTIVE: We investigated the effects of galantamine in patients diagnosed as having AD with cerebrovascular disease (CVD) or probable vascular dementia according to NINDS-AIREN criteria (n=537).

DESIGN: In a multicenter, randomized, double-blind trial, patients received galantamine 24 mg/day (n=359) or placebo (n=178) for 6 months. Primary endpoints were cognition, as measured using the cognitive subscale of the Alzheimer's Disease Assessment Scale (ADAS-cog), and global function, as measured using the Clinician's Interview-Based Impression of Change plus Caregiver Input (CIBIC-plus). Secondary endpoints included assessments of ADL, using Disability Assessment for Dementia (DAD), and behavior, using Neuropsychiatric Inventory (NPI). Patients were monitored for adverse events.

RESULTS: Galantamine showed significantly greater efficacy than placebo on ADAS-cog and CIBIC-plus (both $p \leq 0.001$). For both endpoints, galantamine maintained or improved baseline scores (change ≥0) in more patients than placebo. ADL and behavior were also significantly improved at 6 months compared with placebo and/or baseline (both $p<0.05$?). Galantamine was well tolerated.

CONCLUSIONS: For the first time, we can show a symptomatic therapeutic effect on all key areas of cognitive and noncognitive abilities in patients with AD with cerebrovascular components or probable probable vascular dementia.

Saturday, March 22, 2003

Main Theme Non-Alzheimer Dementias

Sponsored by Novartis Finland

Chaispersons: Maria Spillanti and Matti Haltia

08.30 **Neuropathology in Late-Life Dementias**
Paul Ince, UK

09.00 **Tau Pathologies in Dementing Disorders**
Maria Spillantini, UK

09.30 **Alpha Synucleopathies**
Pekka Jäkälä, Finland

10.00 **Coffee Break**

10.30 **The Clinical Spectrum of Lewy Body Disease**
Ian McKeith, UK

11.00 **Nasu-Hakola Disease**
Matti Haltia, Finland

11.30 **Special lecture**
Effect of Cholinesterase Inhibitors on Non-AD Dementias
Ezio Giacobini, Switzerland

12.00 **Lunch Break**

NEUROPATHOLOGY OF LATE-LIFE DEMENTIA

Professor Paul G Ince

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BACKGROUND: The medical model of dementia syndromes is based on clinicopathological associations in patient groups selected from clinical practice, usually this has implied the study of secondary referral cohorts in the age range 60-80y. Data from older people suggests that the disease categories of Alzheimer's disease, Vascular dementia and Lewy body dementia may be less distinct in the oldest old. This in an important group in society, which will more than double in size in the next two decades, and contributes the great majority of the predicted rise in prevalence of dementia and cognitive decline in western populations.

METHODS: The Cognitive Function and Ageing Neuropathology Study (CFANS) is a longitudinal, prospective, population-based study of cognitive function, psychiatric morbidity and physical health in the elderly. From the 17500+ respondents in the parent study more than 850 have agreed to be brain donors, and 427 brains have been archived. Initial neuropathological evaluation is undertaken using the CERAD protocol and has been followed by more detailed investigation of neurodegenerative and vascular related pathologies, including white matter lesions. Current analysis is based on 209 brains, including 101 demented individuals, and is weighted towards donors aged >80y at death (75%).

RESULTS: Alzheimer-type pathologies (78%) and vascular pathologies (80%) are common. Many demented people satisfy criteria for Alzheimer's disease. However 25% of the sample have pathological data which is either inappropriately high or low compared to their cognitive state, even when all pathologies recorded by CERAD are considered. White matter lesions are common (94%) and deep lesions are independently related to cognitive decline. Molecular pathology suggests that these lesions are vascular in origin.

CONCLUSIONS: Mixed pathology is very common in the oldest old, usually including vascular disease. Pathological substrates do not correlate so closely with cognitive function in this group compared with the 'younger old'.

TAU PATHOLOGY IN DEMENTING DISORDERS

Maria Grazia Spillantini

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Hereditary tauopathies are familial syndromes clinically characterized by frontotemporal dementia and often associated with parkinsonism, oculomotor disturbances or motoneuron signs, they are grouped under the name of frontotemporal dementia and parkinsonism linked to chromosome 17 (FTDP-17). Neuropathologically, tau deposits are found either in neurones or in both neurones and glia and are present in multiple areas of the CNS with a prevalence in the neocortex, hypothalamus, midbrain, pons and spinal cord. The cellular lesions may be indistinguishable from those of Alzheimer disease and sporadic tauopathies such as Pick's disease, progressive supranuclear palsy and corticobasal degeneration. FTDP-17 is caused by exonic or intronic mutations in *Tau*, the gene coding for the microtubule-associated protein tau.

Adult human tau exists as six isoforms generated by alternative splicing. The repeat domain in the carboxy-terminal half, encoded by exons 9 to 12, constitutes the microtubule binding site. Alternative splicing of exon 10, which encodes a 31-amino-acid repeat, gives rise to isoforms with three or four binding domains.

Mutations have been found in exons 1, 9, 10, 11, 12, 13 and in the intron following exon 10, a splice-donor site. Mutations in exon 10 or at the exon 10-intron boundary lead to a pathology in neurones and glia that consists of twisted ribbons predominantly composed of four-repeat isoforms and similar to that found in progressive supranuclear palsy and corticobasal degeneration. Mutations in exons 9, 12 and 13 lead to a predominantly neuronal pathology without a significant glial component. Mutations in exon 11 have shown an important glial accumulation. Some mutations in exon 12 and 13 lead to the formation of paired helical and straight filaments having a morphology indistinguishable from that of the filaments seen in Alzheimer's neurofibrillary tangles and containing all six isoforms like the filaments of Alzheimer disease. By contrast, other mutations cause intraneuronal inclusions, filament morphology and a pattern of tau bands resembling Pick's disease.

The variability of the clinicopathologic phenotypes, may be the result of differing molecular mechanisms. Splice site mutations and some exon 10 mutations alter the splicing mechanism, resulting in the production of only the four-repeat tau isoforms. Other mutations in exon 10 only affect the four-repeat isoforms, while mutations in exons 1, 9, 11, 12 and 13 affect all isoforms; all of them disrupt tau-microtubule interactions and some facilitate tau protein aggregation. The knowledge of tau mutation has helped in producing transgenic mice expressing mutant P301S human tau where tau accumulation is associated with neurodegeneration, indicatiing the direct involvement of tau pathology in cell death and disease development.

α-SYNUCLEINOPATHIES

Pekka Jäkälä, M.D, Ph.D.,

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α-Synuclein is underlying the pathogenesis of common late-onset neurodegenerative diseases, like Parkinson's disease (PD), dementia with Lewy bodies (DLB) and multiple system atrophy (MSA).

PD is defined neuropathologically by the presence of intracellular Lewy bodies and Lewy neurites in the substantia nigra. Point mutations (A53T and A30P) in the α-synuclein gene represent a rare cause of familial PD. However, α-synuclein is the most significant constituent of Lewy bodies in both idiopathic and familial forms of PD. DLB is characterized by α-synuclein immunoreactive Lewy bodies and neurites, especially in paralimbic and cortical areas, whereas in MSA filamentous α-synuclein positive glial cytoplasmic inclusions are found.

In vitro α-synuclein assembles into filaments showing conformation characteristics of amyloid fibres. The A53T mutation increases the rate of filament assembly, indicating that this might be its primary pathogenic effect. In the process of filament assembly/fibrillization, early fibrillization intermediates may be responsible for neuronal death, Lewy bodies and neurites representing the end result in the process. Another possible pathogenic mechanism is oxidative stress. α-Synuclein may have a role in vesicle recycling, the A30P mutation resulting in reduced binding of α-synuclein to vesicles. Thus, impaired neurotransmitter storage arising from mutations in α-synuclein or from α-synuclein accumulation due to oxidative stress could lead to cytoplasmic accumulation of dopamine, which is toxic to dopaminergic neurons. In the normal brain, α-synuclein is concentrated in presynaptic nerve terminals, with no staining in glial cells. In MSA, α-synuclein synthesis may need to be upregulated and/or its degradation rate reduced, to allow accumulation to glial cells.

THE CLINICAL SPECTRUM OF LEWY BODY DISEASE

Ian G McKeith

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Background: Lewy bodies (LB) may be variably distributed in the central and autonomic nervous system and are associated with several different clinical syndromes, predominantly Parkinson's disease (PD), dementia with Lewy bodies (DLB) and primary autonomic failure. The relationships between these syndromes remain largely unresolved.

Method: Review of clinical, neuroimaging and neuropathological studies comparing PD +/- dementia and DLB.

Results: Long term follow up of PD patients finds an increasing prevalence of dementia, up to 57% after 8 years. The majority of these PD dementia (PDD) cases exhibit a fluctuating attentional performance similar to DLB and most fulfil clinical criteria for DLB with extensive cortical LB at autopsy. Recently described differences in α-synuclein positive, neuritic pathology, in the putamen of DLB and PDD, may contribute to clinical characteristics that differ from PD in which these striatal changes are absent. These include postural-instability gait difficulty parkinsonism, present in 85.7% of PDD cases, 62% DLB and 43% PD; diminished levodopa responsiveness in DLB and PDD and increased neuroleptic sensitivity. FP-CIT SPECT and structural MR imaging support these observations.

Conclusions: DLB and PDD share common clinical and pathological characteristics consistent with a LB disease spectrum model in which age is a critical determinant. Future diagnostic criteria and management guidelines should be sensitive to this and discriminate the two diagnostic labels only when it is clinically helpful to do so.

NASU-HAKOLA DISEASE: A FRONTAL DEMENTIA WITH BONE CYSTS

Matti Haltia[1], MD, PhD, Taina Autti[2], MD, PhD, Panu Hakola[3], MD, PhD, Juha Paloneva[4], MD

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Background, patients and methods: Nasu-Hakola disease or polycystic lipomembranous osteodysplasia with sclerosing leukoencephalopathy (PLOSL) is a globally distributed recessively inherited disease. It is clinically characterized by the unique combination of early-onset dementia and bone cysts. The purpose of this paper is to review recent clinical, neuroimaging, neuropathological, and molecular genetic findings in a series of PLOSL patients and to discuss their impact on our views on the molecular pathogenesis of this intriguing disorder.

Results and conclusions: PLOSL usually debuted with pain in ankles and wrists after strain during the third decade, followed by fractures, even after minor accidents, due to cystic lesions in the bones of the extremities. Frontal lobe syndrome and dementia began to develop by age 30, leading to death by age 40. However, some patients did not show any osseous symptoms before the onset of neurological manifestations. At an early stage, neuroimaging disclosed high bicaudate ratios, basal ganglia calcifications, and increased signal intensities of the white matter on T2-weighted MR images. Autopsy findings included advanced frontally accentuated sclerosing leukoencephalopathy with activation of the microglia and microangiopathy. All Finnish patients were homozygous for a loss-of-function mutation of the DAP12 gene while mutations in a second PLOSL gene, DAP12-associated receptor TREM2, were found in a number of non-Finnish patients. The DAP12-mediated signaling pathway, deficient in PLOSL, activates cells of myeloid lineage and seems to play an important role in human brain and bone tissue.

THE EFFECT OF CHOLINESTERASE INHIBITORS ON NON-AD DEMENTIA A COMMON MECHANISM OF ACTION ?

Ezio Giacobini

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Vascular dementia (VD), dementia with Lewy bodies (DLB) and Parkinson dementia (PDD) share some pathological features as well as some symptoms together with a progressive loss of dopaminergic and cholinergic function .The cognitive decline may be related to various degrees of cholinergic deficit which seems to be maximally expressed in DLB (Perry et al, 1990). Patients with Parkinson disease who suffer of dementia (PDD), in addition to typical dopaminergic changes , reveal consistent cholinergic deficits (Nakano and Hirano, 1984 Brake, 1990). Similarly,a reduction in cholinergic markers in the brain of VD patients suggest a cholinergic hypofunction (Maeda, 2002). The results obtained using ChE inhibitors (ChEI) for treatment of all three types of dementia originate from several studies.They provide support to the concept that administration of these drugs may alleviate neuropsychiatric symptoms and stabilize cognition in these patients .Other CNS disorders which may represent indications for ChEI treatment of non-AD dementia are amnesia following traumatic brain injury (TBI), delirium, dementia associated with Down Syndrome, alcoholic dementia and Korsakoff-type amnesic syndrome. Studies are in progress to find out whether or not these disorders may respond to treatment with ChEI. If this is the case, it would suggest a common pharmacological effect for AD as well as for non-AD dementia with memory deficits.

Saturday, March 22, 2003

13.00 **Awards Ceremony**

Main Theme Therapy

Sponsored by Lundbeck

Chaispersons: *Alexander Kurz and Tuula Pirttilä*

13.15 **How to Assess Drug Effects in AD and MCI Using fMRI?**
Serge Rombouts, The Netherlands

13.45 **Current Status of Cholinesterase Inhibitors**
Alexander Kurz, Germany

14.15 **Anti-Glutaminergic Therapies for AD**
Bengt Winblad, Sweden

14.45 **Coffee Break**

15.15 **The Role of The Amyloid Precursor And Environmental Factors In Mediating The Pathological Eeffects of Apolipoprotein E4**
Daniel Michaelson, Israel

15.45 **Treatment of Vascular Dementias**
Ingmar Skoog, Sweden

16.15 **Role of Growth Factors**
Antonino Cattaneo, Italy

16.45 **Closing Remarks**

HOW TO ASSESS DRUG EFFECTS IN AD AND MCI USING FMRI?

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Functional imaging techniques, such as positron emission tomography (PET), and more recently blood oxygenation level dependent (BOLD) functional MRI (fMRI), can be used to monitor effects of drug treatment on brain activation. This presentation will focus on the use of BOLD fMRI for this purpose, particularly in MCI and AD patients. In such studies, one typically applies a (memory) task to activate regions of interest, and tests for changes in brain activation in these regions, comparing fMRI scans between different treatment arms (for example placebo and drug).

A number of issues will be discussed:

- Task development: how to activate regions of interest in MCI and AD. There is a special interest to activate the medial temporal lobe, which is the earliest affected region in AD.
- Study design: the noninvasive character of BOLD fMRI allows multiple fMRI runs within patients (cross over designs) and reproducibility testing.
- Data analysis: methods to test for drug effects on fMRI data.

Recent fMRI studies suggest that drugs that change cholinergic synaptic transmission also show a change in brain activation during certain memory tasks. This has been shown in healthy controls, but also in mild AD patients. In the latter group, enhancement of the cholinergic system results in increased brain activation during memory encoding.

CURRENT STATUS OF CHOLINESTERASE INHIBITORS

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Cholinesterase inhibitors (ChE-I) including donepezil, galantamine, and rivastigmine, are considered to be first line agents for the treatment of patients with mild to moderate dementia in Alzheimer's disease (AD). Efficacy, tolerability, and safety of these compounds have been evaluated in large placebo-controlled clinical trial programmes. These studies have consistently demonstrated significant treatment differences from placebo on cognition, activities of daily living, and global function. As a class effect, ChE-I maintain cognitive ability and activities of daily living at baseline levels for 6 to 12 months. In addition, ChE-I treatment provides beneficial effects on non-cognitive behavioural symptoms and is associated with a reduction in caregiver time. ChE-I are equally effective in patients with moderate and mild dementia in AD. Long-term open-label extension studies have shown that in spite of a gradual deterioration patients on continuous treatment perform better than untreated historical controls. Recently, clinical trials have been completed with donepezil and galantamine in patients with probable vascular dementia and with dementia caused by the combination of AD and cerebrovascular disease. These studies have demonstrated similar effects as seen in AD. In patients suffering from dementia with levy bodies rivastigmine improves cognitive function and non-cognitive behavioural symptoms. Clinical trials with ChE-I are ongoing in patients with mild cognitive impairment who have an increased risk of developing dementia.

ANTI-GLUTAMINERGIC THERAPIES FOR AD

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Treatment regimens throughout the different stages of dementia vary, with objectives broadening as the disease progresses and patients experience a deterioration of their symptoms. In severe dementia, in addition to treating the patient, means of reducing the burden on both the caregiver and health system should be considered. The cost of treating AD is high, and the financial burden increases as the disease progresses. Therapies that delay the institutionalisation of patients will result in significant cost savings. Acetylcholinesterase inhibitors have been approved for the treatment of mild-to-moderate AD. They do not halt the inevitable progression of the disease and as patients deteriorate any therapeutic benefits derived from such medication are liable to decrease the costs. The NMDA-antagonist memantine has been shown to be effective in moderately severe-to-severe AD in one study from Europe and one from US. Additionally, the results from two clinical trials studying the effects of memantine on patients suffering form mild-to-moderate vascular dementia have shown a significantly superior cognitive benefit compared with placebo. While cholinergic agents exert a symptomatic effect by compensating for neuronal loss, memantine prevents neuronal death by inhibiting NMDA receptors that are excessively stimulated in the AD brain. Memantine is also neuroprotective in several animal models of excitotoxicity and neurodegeneration. Data have shown the drug to be safe and well tolerated. Memantine therefore represents a significant addition to the clinician's armamentarium for the treatment of the continuum of AD. Ongoing studies on AD will evaluate the efficacy of combining a cholinergic drug with an NMDA receptor antagonist, since they are both believed to work at different levels of the pathological processes of both AD and VaD. Based on available information, it seems feasible to hypothesize that additive effects may be achieved from such combinations.

THE ROLE OF THE AMYLOID PRECURSOR AND ENVIRONMENTAL FACTORS IN MEDIATING THE PATHOLOGICAL EFFECTS OF APOLIPOPROTEIN E4

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Alzheimer's disease (AD) is associated with several genetic risk factors, of which apolipoprotein E4 (apoE4) is the most prevalent, and with environmental risk factors which include early life education and head injury. In the present study we employed mice transgenic for apoE4 and for apoE3, which is the benign AD isoform, to study the role of the amyloid precursor protein (APP) and its metabolites, and of environmental factors in mediating the neuropathological effects of apoE4. Exposure of apoE3 transgenic mice to an enriched environment elicited improvements in learning and memory, whereas mice transgenic for apoE4 were unaffected by the enriched environment. These effects were associated with activation of synaptogenesis and neurite outgrowth in the hippocampus of the apoE3 but not of the apoE4 transgenic mice. Furthermore, closed head injury experiments revealed that the apoE4 transgenic mice are more susceptible to head injury than the apoE3 transgenic mice, and that this is due to increased mortality of the apoE4 mice and to more effective recovery of the apoE3 transgenic mice following head injury. Further experiments revealed that apoE4 decreases whereas apoE3 increases the levels of secreted APP after head injury. Taken together, these findings suggest that the pathological effects of apoE4 are associated with impairments in neuronal plasticity and repair and that, at least in the head injury paradigm, these effects may be mediated by decreased expression and cleavage of brain APP. The extent to which the pathological effects of apoE4 are also mediated via interactions with other APP metabolites such as β-amyloid will be discussed.

TREATMENT OF VASCULAR DEMENTIA

Professor Ingmar Skoog

Neuropsychiatric Epidemiology Unite, Institute of Clinical Neurosciences,
Sahlgrenska Academy, Göteborg University, Sweden.

Vascular dementia may be caused by several different cerebrovascular diseases, most often infarcts related to stroke and diffuse ischemic white matter lesions. Cerebrovascular diseases may also contribute to the clinical expression of Alzheimer's disease. Treatment strategies for vascular dementia are mainly based on the experience from stroke. It is not clear whether the effect is similar in patients with vascular dementia, or whether the cognitive symptoms are modified by stroke treatment. A patient with stroke-related dementia is often treated with low-dose salicylates. Treatment of risk factors for cerebrovascular disease, such as hypertension, high cholesterol, diabetes mellitus, coronary heart disease, and cardiac arrytmias may prevent new strokes, but it is not clear whether it prevents progression of the cognitive symptoms. Hypertension in patients with vascular dementia (or other dementias) should be treated according to guidelines. Hypertensive demented patients with stroke or white matter lesions should be treated aggressively due to an increased risk of new strokes in these patients. There is no evidence that treatment of hypertension according to guidelines may increase the risk of dementia due to lowering of cerebral blood flow. The Syst-Eur trial suggested that treatment of isolated systolic blood pressure may decrease the risk of dementia, and the SCOPE trial indicated that treatment of mild hypertension in individuals with mild cognitive impairment was associated with less cognitive decline during follow-up. The cognitive component of vascular dementia is most likely amendable to symptomatic treatment with acetylcholinesteras inhibitors, especially in cases with concomitant Alzheimer's disease.

Poster Summary

1

MICROARRAY ANALYSIS OF NONHUMAN PRIMATES: VALIDATION OF EXPERIMENTAL MODELS IN NEUROLOGICAL DISORDERS

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Non-human primates (NHPs) have provided robust experimental animal models for many human related diseases due to their similar physiologies. Nonetheless, there remain profound differences in the acquisition, progression, and outcome of important diseases such as AIDS and Alzheimer's, for which the underlying basis remains obscure. We explored the utility of human high-density oligonucleotide arrays to survey the transcription profile of NHP genomes. Total RNA from prefrontal cortices of human (Homo sapiens), common chimpanzee (Pan troglodytes), cynomolgous macaque (Macaca fascicularis), and common marmoset (Callithrix jacchus), was labelled and hybridized to Affymetrix U95A GeneChip probe arrays. Corresponding data obtained previously from common chimpanzee and orangutan (Pongo pygmaeus) were added for comparison. Qualitative (present or not detected) and quantitative (expression level) analysis indicated that many genes known to be involved in human neurological disorders were present and regulated in NHPs. A gene involved in dopamine metabolism (catechol-O-methyltransferase) was absent in macaque and marmoset. Glutamate receptor 2 was up-regulated and transcription associated genes were down-regulated in NHPs compared to humans. We demonstrated that transcript profiling of NHPs could provide comparative genomic data to validate and better focus experimental animal models of human neurological disorders.

2

GENDER DIFFERENCES IN THE DEPOSITION OF AMYLOIDb IN APPSWE AND PS1 DOUBLE TRANSGENIC MICE

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Transgenic mice carrying both the human amyloid precursor protein (APP) with the Swedish mutation and the presenilin-1 A246E mutation (APP/PS1 mice) develop Alzheimer's disease-like amyloidßprotein (Aß) deposits around 9 months of age. These mice show an age-dependent increase in the levels of Aß40 and Aß42, and in the number of amyloid plaques in the brain. Aß40 and Aß42 levels were measured, andamyloid burden and plaque number were quantified, in the hippocampus at the age of 12 and 17 months in both male and female APP/PS1 mice. In all mice, amyloid burden and plaque number increased markedly with age, with female mice bearing a heavier amyloid burden and higher plaque number compared to male mice,both at 12 months and 17 months of age. The level of Aß40 and Aß42 significantly increased in female mice from 12 to 17 months, and was significantly higher than in the male mice of the same age. Further, there were significant correlations between amyloid burden and Aß42 level in female mice, and amyloid burden and plaques both in female and male mice. Together these data show that female APP/PS1 mice accumulate more amyloid in the hippocampus than age-matched male mice, these results support the hypothesis that gender is a variable in the pathogenesis of AD.

3

AGE-RELATED CHANGES IN THE SYNAPTIC PLASTICITY IN APP+PS1 TRANSGENIC MICE

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Background: Transgenic mice carrying APPswe and PS1 mutations associated with familiar Alzheimer's disease develop amyloid depositions in the hippocampus and cortex, and demonstrate age-related spatial memory impairment. To elucidate the underlying mechanisms, we studied synaptic plasticity by recording long-term potentiation (LTP) in hippocampal slices.

Methods: Male mice, transgenic (TG) and littermate controls (NT), 3 (n = 15) and 17 (n = 21) months old, were used in the study. Field EPSPs evoked by Schaffer collateral stimulation were recorded in the CA1 field in standard 450 µm hippocampal slices. LTP was induced by theta burst stimulation at 100 Hz.

Results: The baseline EPSP slopes were larger in young NT than in young TG mice ($p = 0.003$) but the genotype difference did not reach significance among the aged mice ($p > 0.10$). The stimulation current needed to elicit the maximum population spike free EPSP did not differ between the genotypes. The LTP measured as EPSP slope either 15, 45 or 60 min after the induction, did not differ between the age groups or the genotypes.

Conclusion: The age-related spatial learning impairment in APP+PS1 mice unlikely results from impaired synaptic plasticity in the field CA1 of the hippocampus.

4

INCREASED EXCITABILITY BUT NORMAL IN VIVO LTP IN AGED APP+PS1 TRANSGENIC MICE

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Background: Transgenic mice carrying APPswe and PS1 mutations associated with familiar Alzheimer's disease develop amyloid depositions in the hippocampus and cortex, and demonstrate age-related spatial memory impairment. To elucidate the underlying mechanisms, we studied synaptic plasticity by recording long-term potentiation (LTP) in the hippocampus in freely moving mice.

Methods: Male mice (n = 27), nontransgenic (NT), APP/PS1 double mutant, and APP or PS1 single mutant, 18 months old, were chronically implanted with stimulation electrode in the angular bundle and recording electrodes in the hilus of dentate gyrus. The baseline stimulation current was adjusted to yield a minimal population spike. LTP was induced by theta burst stimulation at 400 Hz. LTP was recording for three days.

Results: Neither the latency to EPSP maximum nor the slope of the I/O curve for EPSP amplitude did not differ between the groups. Both groups carrying the APP transgene often showed a second pop spike 2-3 ms after the first, but this was rare in PS1 or NT mice ($p=0.037$). Also the pop spike to EPSP amplitude ratio was significantly higher in the APP/PS1 and APP mice compared with NT or PS1 mice ($p=0.007$). The EPSP or pop spike enhancement after LTP did not differ between the groups on any testing day (1-3).

Conclusion: The presence of mutated human APP transgene is associated with increased excitability of dentate granule cells. By contrast, amyloid plaques, present only in the APP/PS1 group, did not affect excitability or in vivo LTP.

5

SOMATOSTATIN AND CALRETININ CONTAINING INTERNEURONS IN THE HIPPOCAMPUS OF APP/PS1 TRANSGENIC MICE

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Background. Alzheimer's disease (AD) is characterized by a progressive neuropathology and accompanying decline in cognitive abilities. Typical for AD related neuropathology is its high selectivity for certain cell types. For example, previous reports have shown that hippocampal interneurons containing somatostatin (SOM) are among the most affected cells, whereas calretinin (CR) neurons survive even in cases with severe AD pathology.
Methods. In the present study, we used transgenic mice carrying mutations (human amyloid precursor protein APPswe (APP) and presenilin-1 A246E (PS1) genes), which lead to early onset AD in humans. The animals were perfused and brain sections were immunostained for SOM and CR. The possible SOM and CR colocalization with PSA-NCAM was also investigated using fluorescent-labelled antibodies.
Results. Using a stereological approach we found no significant differences between the transgenic and control mice neither in SOM- nor in CR-immunoreactive (ir) neuron counts in the hippocampus proper. However, transgenic animals had a significantly higher number of CR positive interneurons in the dentate gyrus than their wild-type littermates. Colocalization analyses revealed that SOM but not CR-ir interneurons are colocalized with PSA-NCAM.
Conclusions. SOM and CR interneurons are not affected in this animal model for AD. Interestingly, SOM and CR interneurons in these mice differ from those of rats in respect of colocalization for PSA-NCAM.
Support from EVO (5510) and EU (QLK6-CT-1999-02112).

6

THE SUBCELLULAR DISTRIBUTION OF ESTROGEN RECEPTOR-ALPHA IN THE MOUSE CHOLINERGIC NEURONS IS DEPENDENT ON AGE

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Background. The cholinergic system undergoes severe degeneration in Alzheimer's disease (AD). Previous studies have shown that estrogen has an ability to enhance the function of cholinergic neurons. Here we investigated the effect of estrogen-status on cholinergic neurons in transgenic mice carrying mutations (human amyloid precursor protein APPswe (APP) and presenilin-1 A246E (PS1) genes), which lead to early onset AD in humans.
Methods. The APP/PS1 and control mice were Sham-operated, ovariectomized or ovariectomized and treated with 17-beta estradiol. At the age of 6 and 12 months mice were perfused. Brain sections were double-immunostained for choline acetyltrasferase (ChAT) and estrogen receptor alpha (ERα). ChAT positive neurons were counted in the medial septum-vertical diagonal band (MSVDB) and nucleus basalis of Meynert using a stereological approach.
Results. There were no differences between the treatment and age groups in the total number of ChAT neurons and in the number of ChAT neurons containing ERα. However, the number of ChAT neurons containing nuclear ERα in the MSVDB was higher in 6 months than 12 months old mice. This finding was independent of the treatment and genetical background.
Conclusions. Our finding suggests that ERαs have an age-dependent diffusion rate from cell nucleus to the cytoplasm in the cholinergic neurons of MSVDB. This can have functional significance on the cholinergic neurons, but may also reflect the fact that estrogen levels decrease with age.
Supported by Tekes and Hormos Medical.

7

LONGITUDINAL OBSERVATION ON CSF Ab LEVELS IN APP + PS1 TRANSGENIC MICE

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Background: To achieve an early diagnosis of Alzheimer's disease and monitor the disease progression, considerable attempts have been made to understand the relationship between Aβ42 levels in the cerebrospinal fluid (CSF) and the presence and progression/or severity of the disease. APP+PS1 transgenic mice develop AD-like amyloid pathology around the age of 9 months and spatial memory impairment at a later age; therefore, a longitudinal observation on CSF Aβ levels before and after the appearance of amyloid deposits would provide new insight into the validity of CSF Aβ as a potential biomarker for AD, and also invaluable information on the pathophysiological mechanism related to amyloid deposition.
Methods: Two groups of male APPswe and PS1 doubly transgenic mice (Borchelt et al, Neuron 1997) were used in the present study. CSF samples were taken at the age of 5 and 7 months from the first group; and at the age of 8, 10 and 14 months from the second group. The CSF Aß42 levels were assayed using a commercial ELISA kit (Innogenetics, Belgium).
Results: Our preliminary data shows that at group level there is a continuous, but gradually slowing, increase of CSF Aβ42 levels between 5 and 10 months of age before remarkable Aβ deposits appear in the brain. Interestingly, at the individual level, the increase in the CSF Aβ42 during the two months of follow-up correlated inversely with the initial CSF Aβ42 level, reaching the point of net decrease in some individuals. CSF samples will be taken from the second group at the age of 14 months to see whether the initial increase in CSF Aβ42 levels will convert to a decrease with advancing age.
Conclusions: In APP+PS1 mice, the CSF Aβ42 levels continue to increase until the appearance of marked Aβ deposition.

8

EFFECT OF ESTROGEN ON PLAQUES AND Ab LEVELS IN TRANSGENIC ANIMAL MODEL OF ALZHEIMER'S DISEASE.

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Objective: Recent findings indicate that estrogen could modulate pathology of Alzheimer's disease (AD). Presence of neuritic plaques and amyloid beta (Aß) in hippocampus is of diagnostic and prognostic importance in AD.
Methods: Effects of estrogen on plaque formation and Aß levels in transgenic mice carrying mutations (human amyloid precursor protein APP swe and presenilin-1 A246 genes) that lead to early onset AD in humans were investigated. Non-transgenic littermates were used as controls. Early menopause was mimicked in one group of 7-months old female mice by ovarectomy. Another group received 17-beta estradiol for 90 days beginning at 14.5 months, while the third group was sham-operated. Mice were perfused at 18 months and half of the brain was Bielchowsky silver stained for stereological estimation of plaque numbers in hippocampus. Other half of the brain was used to determine Aß levels with ELISA technique.
Results: Different stages of neuritic plaques were observed in hippocampus of all transgenic mice, majority of which were of the mature type. No significant differences in total plaque counts, plaque morphology or Aß levels were noted between groups. Significant correlation was seen between levels of Aß-40 and Aß-42 and plaque counts.
Conclusion: These findings suggest that estrogen has no effect on plaque counts or Aß load in older transgenic mice.

9

DOPAMINERGIC NEURONAL LOSS AND MOTOR DEFICITS IN CAENORHABDITIS ELEGANS OVEREXPRESSING HUMAN ?-SYNUCLEIN

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Alphasynuclein is a key protein found in synucleopathies including Parkinson's disease. We have created a transgenic *C. elegans* worm that overexpresses human wild type (WT) alphasynuclein or its mutant alanine53 ? threonine (A53T) in its neurons. With different alphasynuclein promoters the expression is either pan-neuronal or targeted to dopaminergic neurons or motor neurons. We have also shown by immunostaining that alphasynuclein is being translated in the respective neurons. There were motor deficits when WT or A53T alphasynuclein was expressed pan-neuronally or in motor neurons. Neuronal and dendritic loss was accelerated in all *C. elegans* dopaminergic neuron sets when alphasynuclein was overexpressed under control of a dopaminergic neuron- or pan-neuronal- promoter. There were no significant differences in neuronal loss between WT and A53T forms or whether the worms were scored at 5d of at 2 weeks. This transgenic model in C. elegans may provide insights to pathophysiological mechanisms of various synucleopathies including PD.

10

A30P a-SYNUCLEIN MUTATION MICE AS A MODEL OF PD AND OTHER DISEASES WITH LEWY BODIES

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Background. Recently two point mutations A50T and A30P in the α-synuclein gene were found to be a cause of familial Parkinson's disease. α-Synuclein is the prime suspect for contributing to Lewy pathology in Parkinson's disease (PD) and dementia with Lewy bodies (DLB). α-Synuclein inclusions are also found in multiple system atrophy (MSA).

Methods. We developed a transgenic mouse line carrying the human A30P α-synuclein mutation to see whether overexpression of human mutated α-synuclein would lead to neuropathological, neurochemical and behavioural characteristics of these neurodegenerative diseases. Immunohistochemical stainings and western blotting were done by anti α-synuclein antibodies. Behavioral testing included Shirpa-test battery, Rotarod, beam walking, OF, water maze and RAM.

Results. At 12 months of age, heterozygous mice expressed intracellular α-synuclein accumulation in the cortical layer IV, the hippocampus, striatum and substantia nigra. In the open field test, heterozygous mice did not differ from the controls, but homozygous mice showed hypoactivity and reduced exploratory behaviour at the age of 8 months.

Conclusions. The α-synuclein A30P transgenic mice show some attentional and motor deficits similar to other PD models, such as mice treated with the toxin MPTP. It remains to be seen whether the observed symptoms are progressive with aging.

11
CNS EXPRESSION OF DAP12/TREM2 – THE MOLECULES BEHIND PLOSL

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Background PLOSL (Nasu-Hakola disease) is a recessively inherited disease characterized with systemic bone cysts and early-onset dementia. Our group has recently characterized the molecular background of PLOSL by identifying mutations in *DAP12* and *TREM2* genes (Paloneva J, et al. 2000 & 2002). The encoded proteins form an activating signal transduction complex in myeloid cells, but their function in brain and bone is unknown. To understand how DAP12/TREM2 deficiency leads to PLOSL, we have compared their spatiotemporal expression in mouse, characterized the CNS cell types expressing them, and followed their intracellular trafficking.
Methods and Results Analyses of transcripts in mouse brain indicate that DAP12 and TREM2 are expressed from E17 to adulthood, with a highly comparable distribution. Interestingly, in primary cell cultures DAP12 and TREM2 are exclusively expressed in microglia and oligodendrocytes. Overexpression experiments demonstrate that both, DAP12 and TREM2 travel via Golgi-network to the cell membrane, where they co-localize partially. In polarized cells DAP12 and TREM2 get apically targeted.
Conclusions DAP12/TREM2 mediated signal transduction is essential for normal brain function. Here we show that DAP12 and TREM2 follow similar spatiotemporal expression pattern in mammalian brain, and that microglia and oligodendrocytes are the major DAP12/TREM2 expressing cells of CNS. Further characterization of the intracellular targeting and molecular interactions of DAP12/TREM2 will provide new insights into the pathogenesis of this inherited dementia.

12
CLEAVAGE OF THE CYCLIN-DEPENDENT KINASE 5 (CDK5) ACTIVATOR P35 TO P25 DOES NOT INDUCE TAU HYPERPHOSPHORYLATION

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Background. Hyperphosphorylated tau protein is the primary component of neurofibrillary tangles observed in several neurodegenerative disorders. It has been hypothesized that in certain pathological conditions, the calcium activated protease, calpain, would cleave the cyclin-dependent kinase 5 (cdk5) activator p35 to a p25 fragment, which would lead to augmented cdk5 activity, and cdk5-mediated tau hyperphosphorylation.
Methods. To test the above-mentioned hypothesis we studied the relationship between p25 production, cdk5 activity, and tau phosphorylation in rat hippocampal neuronal cultures treated with glutamate, NMDA, and calcium ionophores to induce calpain activity. The levels of p35, p25, and phosphorylated tau were studied by immunoblotting, whereas cdk5 activity was assessed by a standard kinase assay.
Results. In glutamate treated cells p35 was cleaved to p25, and this was associated with elevated cdk5 activity. However, tau phosphorylation was concomitantly decreased at multiple sites. The calpain inhibitor MDL28170 prevented the cleavage of p35 but had no effect on tau phosphorylation, suggesting that calpain-mediated processes, i.e. the cleavage of p35 to p25 and cdk5 activation, do not contribute to tau phosphorylation in these conditions. Treatment of the neuronal cultures with N-methyl-D-aspartic acid (NMDA) or with calcium ionophores resulted in an outcome highly similar to that of glutamate.
Conclusions. We conclude that, in neuronal cells, the cleavage of p35 to p25 is associated with increased activity of cdk5 but not with tau hyperphosphorylation.

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INTERACTION OF AGGREGATED AMYLOID BETA WITH TRITON-SOLUBLE PROTEINS OF SH-SY5Y NEUROBLASTOMA CELLS

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Background. The neuritic plaques are mainly constitued of amyloid fibrils. The fibrillar amyloid beta (fAß) has been associated with neuronal dystrophy and both in vitro and in vivo neurotoxicity. Conversion of Aß to fAß increases his binding to membrane proteins. However, even if some membrane proteins have been shown to bind fAß, the mechanism of its internalization and neurotoxicity is still largely unclear. Recent data have proved that some amyloid-surface binding molecules (ASBiM) could protect neurons from fAß toxicity, probably by disturbing the fAß-membrane protein(s) interaction. The aim of the present study is to identify the proteins involved in this interaction.

Methods. Our approach consists of (i) extracting membrane proteins by detergents from neuroblastoma cells SH-SY5Y; (ii) selecting fAß binding proteins, by co-precipitation of the detergent-soluble proteins either with fAß or with a mix of fAß and ASBiM; (iii) analyzing by electrophoresis the pattern of co-precipitated proteins; (iv) identifying by mass spectrometry the proteins, in particular proteins whose interaction with fAß is disturbed by addition of ASBiM.

Results. Preliminary results have shown that it is possible to analyze by 1D and 2D electrophoresis a large number of proteins co-precipitated with fAß. Their identification will be soon carried out.

Conclusion. This work would allow a better understanding of the action of the ASBiM, which would help to define their use in the treatment of Alzheimer's disease.

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INTERACTION OF ?-AMYLOIDS WITH CELL MEMBRANE PROTEINS AND SIGNALIZATION

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Background. Polymerization of ß-amyloid peptides (Aß) has been identified as a major feature of the pathogenesis of Alzheimer's disease [1,2]. As Aß assembles into oligomeric forms (diffusible clusters and fibrils), it possesses high neuronal toxicity [3,4]. According to a series of experiments (partly performed in our laboratory) the interaction of aggregated Aß-peptides with the cell membrane proteins plays pivotal role in the neurotoxicity of these peptides. Some short peptide fragments prevent neurons from the toxic effect of Aß-peptides.

Methods. We have studied different Aß peptides and analogs in 10µM concentration using the MTT-test (formazan-test) on SH-SY5Y human neuroblastoma cells for measuring the neurotoxicity. In another experiments we have studied the neuroprotective effects of short peptide fragments and peptide analogs in the same test. Short peptides were used in 20 µM concentration.

Results. The aggregation of Aß peptides shows good correlation with their toxicity in MTT test (Table 1.)

Table 1. Aggregation and toxicity of amiloid peptides

SEQUENCE	AGGREGATION	TOXICITY
1. Aß 1-40	+++	+++
2. Aß 1-42	++++	++++
3. Aß 25-35	+++	+++
4. Aß 31-35	+++	+++
5. all-D- Aß 1-40	+++	+++
6. reverse Aß (42-1)	—	—
7. reverse Aß (35-25)	—	—

Most of the short peptide fragments proved to be neuroprotective in MTT test. However, dimer peptides were neurotoxic (Table 2.)

Table 2. The role of integrins –protecting effects of RGD analogs (MTT-test, SH-SY%Y cell culture)

PEPTIDE		PROTECTING EFFECT
1. GRGDS	+ Aß 1-42	no protection
2. GRADS	+ Aß 1-42	++++
3. Ac-homo RGD	+ Aß 1-42	++++
4. GRGES	+ Aß 1-42	++++
5. GRGD-ßA	+ Aß 1-42	++++
6. $(GRGD\text{-}ßA)_2Lys$ dimer	+ Aß 1-42	toxic!
7. $(CRGDC\text{-}ßA)_2Lys$ dimer	+ Aß 1-42	toxic!

Conclusion. According to our hypothesis clusterization of integrin receptors start the neurotoxic cascade caused by Aß-peptides.

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ANTI-INFLAMMATORIC EFFECT OF ESTROGEN RECEPTOR ANTAGONISTS IN MICROGLIAL CELL CULTURE MODELS.

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Background: In recent years, inflammatory mechanisms have been increasingly appreciated as important steps in the pathology of Alzheimer's disease (AD). Microglia, the resident macrophages of the brain, have been reported to be pathologically activated in AD. Activated microglia can secrete proinflammatory molecules including cytokines, chemokines, *etc.* which can contribute to progressive neuronal damage. It has been shown that estrogen receptors regulate inflammation in different cell systems. The purpose of this study was to compare the anti-inflammatoric effect of different estrogen receptor antagonists against lipopolysaccharide(LPS)-induced inflammation in microglial cells.

Methods: We used LPS activated primary rat microglia, mouse N9-microglial cell line or primary rat hippocampal slices to study the effect of 17-β-estradiol or estrogen receptor antagonists Tamoxifen, 4-hydroxy-tamoxifen, Raloxifen and ICI 182.780. Induction of inflammation was characterized by determining nitric oxide and IL-6 concentrations from cell culture media. The viability of cultures was quantified by measuring lactate dehydrogenase activity.

Results: Tamoxifen, 4-hydroxytamoxifen, Raloxifen and ICI 182.780 decreased the LPS-induced inflammatory response in primary rat microglial cells and in mouse N9-cell line, but not in primary hippocampal slices. However, 17-β-estradiol did not provide any protection.

Conclusions: These observations show that in our microglial cell culture models 17-β- estradiol is not anti-inflammatorial unlike many estrogen receptor antagonists are.

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THE NEUROPROTECTIVE POTENTIAL OF ESTROGENS, PHYTOESTROGENS AND SERMS AGAINST OXIDATIVE STRESS

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Background: Beta amyloid (Aß) is a neurotoxic peptide that accumulates in the brains of AD patients. Oxidative stress may play a key role in its toxic actions. Estrogens and estrogen-like compounds have been shown to be neuroprotective in CNS and it has been suggested that they can protect neurons against Aß and oxidative stress induced cell death. The major goal of this study was to compare the neuroprotective potential of 17ß-estradiol (E2), estrone (E1), tamoxifen (Tam), 4-OH-tamoxifen (OH-Tam), diethylstilbestrol (Des) and genistein (Gen) in two different models for oxidative stress induced cell death: in rat primary hippocampal neurons and in differentiated human neuroblastoma cells (d-SH-SY5Y).

Methods: Neuronal cell death was induced either by hydrogen peroxide (H_2O_2) or $Aß_{1-42}$, the toxic fragment of Aß protein. The neuronal cell death was determined by measuring the activity of lactate dehydrogenase (LDH), which is released upon cell damage. The expression level and phosphorylation status of tau was determined by Western blotting.

Results: Pre-treatment with nanomolar concentrations of E2, OH-Tam and Des for 24 h was neuroprotective against H_2O_2-induced cell death in hippocampal and d-SH-SY5Y cultures. E2 decreased the LDH release approximately 40% in primary cultures whereas the decrease induced by Des and OH-Tam was 20% and 30%, respectively. In d-SH-SY5Y cultures the E2-induced decrease was 30%. In addition, these compounds appeared to be neuroprotective against $Aß_{1-42}$ in primary neuron cultures. Further, H_2O_2 triggered change in soluble tau levels was affected by E2.

Conclusions: In this study, the neuroprotective potential of the studied compounds increase in the following order Des<OH-Tam<E2. E2 is the most potent neuroprotector at nanomolar concentrations.

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ESTROGEN ROBUSTLY REGULATES HIPPOCAMPAL PLACE CELLS.

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Background. Estrogen has a strong influence on hippocampal synaptic activity and even morphology (Woolley et al. J Neurosci 17, 1997). However, as yet there is little direct evidence that this regulation affects hippocampal information processing.
Methods. In order to examine how hippocampal encoding varies with estrogen treatments, we studied the activity of hippocampal CA1 place cells as Wistar rats were exposed alternately to a familiar and a novel environment. In total, 205 pyramidal cells were recorded from 5 young male rats, and 5 groups of female rats: 3 young ovary-intact , 3 young ovariectomized , 5 aged ovary-intact , 7 aged ovariectomized , and 6 aged ovariectomized with estradiol injections.
Results. The stability of place fields within the two environments did not differ due to estrogen status or to sex. On the other hand, in examining the response of place fields to the changes in environment, we found that males and females lacking estrogen (ovariectomized) tended to produce new spatial representations for the new environment, whereas the sham-operated females and those ovariectomized with estradiol-injections maintained similar representations for the two arenas.
Conclusions. In sum, low estrogen levels may facilitate hippocampal encoding of new spatial information. High estrogen levels may facilitate pattern completion towards previously learned information.

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EXPRESSION OF CLUSTERIN/APOJ - A SECRETED b-AMYLOID BINDING CHAPERONE - IS INDUCED BY PROTEIN ACETYLATION.

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Background: Clusterin/ApoJ is a multifunctional glycoprotein present in senile plaques and cerebrovascular amyloid depositions. Its function is still elusive but e.g. a role in complement regulation, lipid transport and chaperone functions have been proposed. Expression of clusterin is sensitive to environmental changes, such as DNA methylation and heat-shock stress. Environmental insults affect the acetylation status of histones and transcription factors. Our purpose was to study whether trichostatin A (TSA), an effective inhibitor of class I and II histone deacetylases and hence a selective inducer of protein/histone acetylation, can affect the expression of clusterin.
Methods: Several mouse and human neuroblastoma cell lines and primary astrocytes were exposed to TSA. The expression of clusterin in cultured neural cells was assayed by Northern hybridization and Western blotting. Expression of APP mRNA was analyzed by Northern assay.
Results: TSA treatment highly upregulated the clusterin mRNA expression in human SH-SY5Y, IMR-32 and SK-N-AS neuroblastomas, astrocytes and retinal pigment epithelial cells. Expression was at the highest level 24 h after treatment. Western blotting confirmed the upregulation of clusterin-β expression, especially that of glycosylated 64 kDa isoform. TSA exposure did not affect APP mRNA expression.
Conclusion: Clusterin expression is a sensitive marker of environmental stress and its role, either neuroprotective or neurodegenerative, needs further studies.

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APOLIPOPROTEIN E COLOCALISES WITH BUT DOES NOT INDUCE NEUROFIBRILLARY TANGLE-LIKE STRUCTURES IN NEURONAL CELL LINES

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Background. Recently, a hypothesis was proposed linking amyloid pathology to the formation of neurofibrillary tangles through the major genetic risk factor for sporadic late-onset Alzheimer's disease, apolipoprotein E (apoE) (Huang, et al. (2001) PNAS. 98, 8838). Beta-amyloid was reported to induce carboxyl-truncation of apoE, in particular apoE4 and truncated apoE4 fragments were reported to induce neurofibrillary tangle formation by direct association with tau. We investigated this hypothesis.
Methods. Full-length or carboxyl-truncated apoE were expressed normally, or cytosolically, in Neuro2A and PC12 cells. Expressed apoE was FLAG or Cycle3GFP tagged to investigate the effects of tagging on apoE function. ApoE fragmentation in the presence of beta-amyloid was determined by Western blotting. The intracellular distributions of tau, neurofilament proteins and apoE were determined by immunofluorescence.
Results. Treatment of Neuro2A cells expressing full-length apoE with beta-amyloid induced minor carboxyl-truncation of apoE. However, we did not observe any colocalisation of apoE with tangle-like structures. Cytosolically expressed carboxyl-truncated apoE was found to colocalise with, but not induce, endogenous tangle-like structures that were composed of neurofilament proteins rather than tau. Amino-terminal tagging of apoE with green fluorescent protein, as in previous studies, resulted in formation of artefactual inclusions.
Conclusions. Our data indicate that carboxyl-truncated apoE fragments do not play a major role in the formation of neurofibrillary tangles.

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VP22-BASED PROTEIN DELIVERY SYSTEM IN NEURONAL CELL CULTURES.

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Background. Our goal was to study genes which are regulated by FOXO3a forkhead transcription factor in primary neurons by means of array hybridizations. However, the efficiency of plasmid transformation for primary neurons is very low with current methods (around 1% in fact). Therefore we decided to use VP22-based Voyager system for protein delivery (Invitrogen). The Voyager system theoretically can translocate recombinant protein into virtually 100% of the cells in a culture.
Methods. We constructed a series of plasmids expressing the N-terminal VP22 fusion proteins containing human HA-tagged FOXO3a genes (both wild type and deficient in phosphorylation mutated variant), Red Fluorescent Protein gene, C-terminal c-myc epitope tag and C-terminal polyhistidine (6xHis) tag. The functionality of vectors was evaluated in cell cultures by different biochemical and immunochemical methods.

Results. We found that the VP22 fusion proteins can efficiently translocate between different cell lines. We also found that the FOXO3a transcription factor is active in our constructs although it is fused to VP22 and other large proteins on both N and C termini. FOXO3a (in case of mutant variant) can efficiently block cell proliferation in various cell lines. It can also regulate the expression of a target gene on a reporter plasmid.

The investigations are currently underway to study whether the translocation can occur in primary neurons.

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CAJAL-RETZIUS CELLS IN ALZHEIMER'S DISEASE AND NORMAL AGING

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Background. Neurodegeneration in Alzheimer´s disease (AD) is associated with aberrancies in synaptic plasticity and neuronal repair. Several trophic molecules that play key roles during normal embryonic and fetal brain development are re-activated during the course of the disease. One of them is the large glycoprotein Reelin that is secreted by Cajal-Retzius cells (CRs) located in layer I. Reelin assures correct layering of the neocortex acting on the downstream target protein Disabled-1. Disturbances of this pathway interfere with cytoskeletal hyperphosphorylation, presenilin and amyloid precursor protein signalling as well as Cdk-5 mediated mechanisms known to be involved also in the pathogenesis of AD.

Methods. Immunohistochemistry for Reelin and several cytochemical markers were used to characterize CRs in the hippocampal formation of AD and controls.

Results and conclusions. Analysis showed that CRs are preserved in AD. They maintain their morphological heterogeneity in layer I of entorhinal cortex in the normal senescent brain and in AD. Few Reelin-immunoreactive CRs displayed immunoreactivity for paired helical filaments - typical for pyramidal cells in AD. Co-localization of beta-Amyloid and Reelin was not revealed. The facts that CRs are preserved in AD and that the majority of them displays no signs of degeneration indicates normal function.

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BODY MASS INDEX ASSOCIATED WITH MILD COGNITIVE IMPAIRED WOMEN

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Background: Alzheimer's disease (AD) is often accompanied by weight loss and low body mass index (BMI).

Methods: The purpose of this study was to investigate the BMI between a group of 19 cognitively normal women and another group of 43 women with mild cognitive impairment (MCI) - a transition state between normal aging and dementia.

Results: MCI women had a higher BMI compared with control women ($p<0.05$) and there was a significant association between BMI and MCI even after adjustment for confounders including the ApoE e4 allele. However, MCI e4 homozygotes had a lower BMI and weight than heterozygotes.

Conclusions: Our data suggest that high BMI is associated with MCI, while APOE e4/4, a high risk for AD, is associated with a low BMI in MCI women. Thus, the maintenance of a reasonable BMI could be of importance in reducing the risk of MCI and preventing the developing of AD.

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METABOLIC SYNDROME IN ALZHEIMER'S DISEASE: A POPULATION-BASED STUDY

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Background: To determine the association between operationally defined metabolic syndrome and probable or possible Alzheimer's disease in an elderly population.
Methods: 762 elderly non-diabetic subjects aged 69 to 78 years. Main outcome measures: presence of metabolic syndrome according to the definition of the National Institute for Health and diagnosis of probable or possible Alzheimer's disease (AD).
Results: 30 subjects were classified as having probable or possible AD, and 262 subjects as having metabolic syndrome. Metabolic syndrome was more frequently detected AD patients than in control subjects (OR 95% CI; 2.60: 1.24 to 5.44). However, when men and women were studied separately this was detected in women (CI; 5.87: 1.92 to 17.96) but not in men (CI; 0.73: 0.15 to 3.45). We also studied the association in Apoe E4 carriers and non-carriers. 29.0 % of the controls and 53.3 of the AD patients were Apoe E4 cariers ($p<0.01$). Frequency of AD did not differ by the presence of metabolic syndrome in Apoe E4 carriers (OR; 1.27: 0.44 to 3.64), but in the Apoe E4 non-carriers there was a clear difference between the groups (OR; 7.12: 1.96 to 25.87).
Conclusions: Metabolic syndrome is more frequently found in patients with AD than in the non-demented control population. We found this in women only, which may be due to selective survival.

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MIDLIFE ALCOHOL CONSUMPTION AND RISK OF MILD COGNITIVE IMPAIRMENT IN LATE-LIFE: A POPULATION-BASED STUDY

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Background: There are only few studies concerning alcohol consumption and the development of cognitive impairment. Recent studies suggest that moderate alcohol consumption may have a protective effect on dementia and cognitive impairment. The aim of this study was to evaluate the relation of midlife alcohol consumption and mild cognitive impairment (MCI) in a Finnish population.
Methods: The participants were derived from random population-based samples previously studied within the framework of the North Karelia Project and the FINMONICA study in 1972, 1977, 1982 and 1987, when their baseline alcohol consumption was assessed. The average follow-up time was 21 years. Cognitive status was assessed in the re-examination in 1998 when the subjects who scored 24 or less in the MMSE were addressed for further diagnostics for MCI.
Results: One third of the population did not use alcohol at midlife. Beer and liquor were both used by one fourth of the subjects and wine by 16 %. Subjects who never consumed alcohol at midlife, as well as those who consumed once a month or more frequently, had twice as high MCI risk in late-life than those who consumed alcohol less frequently. Drinking wine at midlife was associated with an increased risk of MCI.
Conclusions: Never using alcohol as well as frequent alcohol use at midlife, are both risk factors for MCI later in life. These results suggest a U-shaped relationship between alcohol consumption and the development of MCI.

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ESTROGEN REPLACEMENT THERAPY IS ASSOCIATED WITH SUPERIOR MEMORY IN OLDER WOMEN

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Background. The aim of this study was to investigate the possible effect of estrogen replacement therapy (ERT) and apolipoprotein E (APOE) genotype on the cognitive state of healthy elderly women.
Methods. The study group included 475 healthy elderly women from a population-based random sample (n = 1150) of the city of Kuopio, Finland. Subjects (60-76 years of age) were evaluated using neuropsychological tests and a structured interview. A blood sample was taken for APOE genotype determination.
Results. Women who had used ERT showed significantly better performances in the immediate and delayed episodic memory tasks. The presence of the APOE ε4 allele or ERT and APOE genotype interaction had no association with the cognitive performance.
Conclusions. This study demonstrated that the ERT use may help to maintain memory in healthy elderly women and this effect appears to be independent of the APOE genotype.

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A 90-YEAR-OLD MONOZYGOTIC FEMALE TWIN PAIR DISCORDANT FOR ALZHEIMER'S DISEASE

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Background: The prevalence of Alzheimer's disease (AD) increases exponentially with advancing age. The concordance of AD is higher in monozygotic than in dizygotic twins. The effect of gene-environment interaction on the disease expression can be studied with twins.
Methods: PET, MRI, and neuropsychological findings are described in 90-year-old monozygotic female twins, who have remained discordant for probable AD for at least seven years. The reference group consisted of 7 women and 3 men aged 73 ± 3 (mean ± SD). Twins' life histories were remarkably similar, except for continuous NSAID use of the unaffected twin for decades.
Results: The regional cerebral glucose metabolic rates and all but two CERAD test scores of the affected twin were more than 2 SD below the reference group's mean values. These values were normal in the unaffected twin. The affected twin had moderate hippocampal and temporoparietal atrophy, whereas the hippocampi were intact, and cortical atrophy mild in the unaffected twin.
Conclusions: These twins were discordant for AD, although they had reached the age in which the prevalence of AD is high among women. It is of interest, that the unaffected twin had had a long history of anti-inflammatory medication.

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DELUSIONAL IDEATIONS IN ALZHEIMER'S DISEASE

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Background: Psychotic features in Alzheimer's disease (AD) are common phenomena. We still have a poor understanding of the natural course of these symptoms and their biologic correlates.
Methods: A retrospective analysis of the medical charts of 129 consecutive patients (80 female, mean age: 76.8±5.4 yrs, mean MMSE 16.4±4.3) diagnosed as having probable AD was performed. The presence of delusions was assessed by clinical interview based on the Neuropsychiatric Inventory powered by a series of specific questions addressing other types of delusional ideations which are not mentioned in NPI (including those typical for schizophrenia).
Results: Delusions of any type were present in more than 60% of patients; age, education, and gender were not significant predictors. Frequency of delusions versus dementia severity analysis revealed the existence of two clusters of delusions: we called them, respectively, memory-related (e.g. things are being stolen or delusional misidentifications) and memory-neutral (like persecutory or grandiose delusions). The rate of the latter was low and not linked to dementia severity. On the other hand, memory-related delusions were much more widespread and occurred mainly in moderate stage of dementia (a Ç-shaped correlation between their frequency and MMSE score).
Conclusion: The profile of delusions in AD is different from that seen in schizophrenia, further supporting the hypothesis that AD-associated psychosis is a distinct phenomenological syndrome. Should we use different therapeutic strategies for memory-related delusional ideations (cholinesterase inhibitors?) needs to be further elucidated.

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RELATIONSHIP BETWEEN DEMENTIA AND PLASMA LEVELS OF Aβ40 AND Aβ42 IN PATIENTS WITH DOWN SYNDROME – A PROSPECTIVE LONGITUDINAL STUDY

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Background. Patients with Down Syndrome (DS) develop neuropathological changes consistent with Alzheimer's Disease (AD) by the 40 years of age possibly due to the increased APP gene dosage.
Methods. Nine demented and 21 nondemented DS patients were included in the study. Neuropsychological evaluation and blood tests were done yearly during 5-year period. Concentrations of Aβ40 and Aβ42 were measured by ELISA. APOE genotype was determined and neuropathological analyses were done in 11 patients who died during the follow up.
Results. Plasma levels of Aβ42 were higher in both groups than in general population. There were no significant differences between the demented and non-demented groups. Aβ40 levels were lower than Aβ42 in both groups. Six patients became demented during the study but there was no relationship between Aβ levels and development of AD. There was no correlation between plasma Aβ levels and APOE genotype or amyloid pathology in brain.
Conclusions. The effect of APP overexpression in DS patients is reflected in blood. Plasma Aβ does not contribute to the development of dementia and amyloid pathology in the brain in DS patients.

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ENTORHINAL CORTEX IS MORE ATROFIED THAN HIPPOCAMPUS IN MCI SUBJECTS

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Background: Mild cognitive impairment (MCI) is a transition state between normal aging and dementia.
Methods: We measured the volumes of the hippocampus and ERC from MR images in 59 controls, 65 subjects with MCI and 48 patients with AD. The groups were matched for age and gender. The MCI subjects and controls derived from population based cohorts.
Results: Volumes of the hippocampus and ERC were significantly decreased (C>MCI>AD). The most efficient overall classification between controls and MCI subjects was achieved with ERC measurements (65.9%, sensitivity 66.2%, specificity 65.5%). Instead between both, controls and AD patients, and MCI subjects and AD patients the best overall classification was achieved with hippocampal measurements (90.7% and 82.3%, respectively).
Conclusions: The ERC is more atrophied than hippocampus in MCI subjects and it serves best in distinguishing MCI subjects from controls. The hippocampal volume is a better marker than ERC volume in distinguishing AD patients from controls and from MCI subjects.

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VOXEL-BASED MORPHOMETRY IN CONTROLS AND PATIENTS WITH ALZHEIMER'S DISEASE INCORRECTLY CLASSIFIED BY HIPPOCAMPAL VOLUMETRY

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Background: Atrophic hippocampi as detected by volumetry are a sensitive marker of Alzheimer's disease (AD), but some overlap exists with non-demented controls. Aim of this study was to test whether voxel-based morphometry (VBM) with Statistical Parametric Mapping (SPM) can detect atrophy in AD patients overlapping with controls based on hippocampal volumes.
Methods: Seven patients with AD and 11 controls with similar normalized hippocampal volumes underwent two separate VBM comparisons to detect regions more atrophic in AD than in controls and *vice versa* using SPM99. Significance threshold was set at $p<0.001$, and age and intracranial volume were included as covariates.
Results: VBM detected atrophy bilaterally in the temporoparietal and cingulate cortices, precuneus, and medial temporal regions despite the reduction of total gray matter volume was not significant (5.0 %). Hippocampal atrophy was detected on the left side only, centered in the hippocampal body. When compared to AD patients, controls showed a restricted area of apparent gray matter reduction centered in the straight sinus but no neocortical atrophy.
Conclusion: VBM might be more sensitive than hippocampal volumetry in detecting medial temporal atrophy in AD.

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RECOGNITION OF NOVEL OBJECTS AND NEW ARRANGEMENT OF OBJECTS DIFFERENTIALLY ACTIVATES THE MTL SUBAREAS IN HUMANS

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Background: A number of previous animal studies have reported a distinction between the contributions of the hippocampus and perirhinal cortex to memory, such that the hippocampus is crucial for spatial memory, while the perirhinal cortex plays a pivotal role in visual recognition memory. The literature on human neuropsychology, however, is sparse on this issue.
Methods: To determine if such a distinction is also present in humans we conducted a functional magnetic resonance imaging (fMRI) study, comparing the medial temporal lobe responses to visually presented changes in object identity or spatial arrangement of objects under same experimental conditions in 12 young healthy control subjects.
Results: The anterior hippocampus, and perirhinal cortex participated in object novelty recognition, whereas the posterior hippocampus was involved in detection of new spatial arrangement of familiar objects. The average of parahippocampal responses were rather similar in both conditions.
Conclusions: A functional double dissociation also exists in the human between perirhinal cortical encoding of object novelty and hippocampal encoding of new spatial arrangements. Furthermore, a robust specialization within the hippocampus along its long axis was observed, a finding that may explain some previous controversies in primate studies about its role in object recognition.

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MEDIAL TEMPORAL LOBE ACTIVATION DURING ENCODING AND CUED RETRIEVAL OF WORD-PICTURE PAIRS

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Background: Although the involvement of medial temporal lobe (MTL) substructures in episodic memory has been indicated, it still remains unknown whether their contribution comprises formation, storage or retrieval of memories. Functional magnetic resonance imaging (fMRI) is a good method to investigate episodic memory in humans *in vivo*.
Methods: To examine the MTL activation areas during encoding of novel word-picture pairs and their cued retrieval, 15 young healthy subjects were studied with fMRI using blood-oxygen-level-dependent (BOLD) contrast.
Results: In the encoding-baseline comparison, activation was detected in the head of the hippocampus and parahippocampal gyrus bilaterally. The retrieval-baseline contrast yielded a small activation area in the left hippocampus, located posterior to the hippocampal activation area during encoding. The direct encoding-retrieval comparison revealed bilateral activation in the parahippocampal gyrus.
Conclusions: These findings support the conception that MTL substructures have different roles during encoding and retrieval of new information. The hippocampus and parahippocampal gyrus are consistently activated during successful encoding of novel information while their contribution to retrieval requires further studying.

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INVOLVEMENT OF THE ROSTRAL LIMBIC SYSTEM IN FRONTOTEMPORAL DEMENTIA

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Background: Some brain structures demonstrated to be atrophic in frontotemporal dementia (FTD) belong to the rostral limbic system (RLS), devoted to the tuning of adaptive behavior after assessment of the motivational content of internal and external stimuli.
Aim of this study was to evaluate whether FTD can be a neural system disease caused by dysruption of the RLS.
Methods: Nine patients with FTD (MMSE 15±9) and 26 healthy controls (MMSE 29±1) underwent high resolution 3D brain magnetic resonance imaging. SPM99 algorithms performed a) spatial normalization to a Talairach-based template, b) segmentation and c) smoothing of the gray matter. Significance threshold was set at $p<0.05$ corrected for multiple comparisons.
Results: Five regions of the RLS were atrophic at $p<0.05$ corrected (anterior cingulate, mesial orbitofrontal and anterior insular cortices, amygdala, and ventral striatum). The periaqueductal gray showed atrophy at $p<0.001$ uncorrected.
Conclusions: All the regions of the RLS were atrophic in this FTD sample. Damage to this system can explain most of the syndrome of FTD: inappropriate social and personal conduct, lack of insight, impairment of executive functions, stimulus-bound behaviors, emotional blunting, aspontaneity and economy of speech, stereotypy and perseveration, dietary changes, and grasping.

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EXTENSIVE LINKAGE DISEQUILIBRIUM WITHIN THE *CYP19* GENE REGION ON CHROMOSOME 15q21

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Background: We previously found that late onset Alzheimer's disease (AD) was associated with genetic marker located on 15q21.1 near the *CYP19* gene encoding the aromatase enzyme. To assess the suitability of *CYP19* gene region for case-control association analysis, we evaluated the extent of linkage disequilibrium (LD) at this region.
Method: We genotyped nine single nucleotide polymorphisms (SNPs) within *CYP19* region (111 kb) and calculated D` values for SNP pairs. We also carried out four gamete test with the aide of pair-wise haplotype estimation analysis to identify haplotype blocks.
Results: Using the standardized D' value of 0.30 as the significant cut off value for LD, 75% of SNP pairs were found to be in LD. When allowing for small proportion of recombination (< 3%) to occur between two loci, only two haplotype blocks defined by SNPs 2-4 and 5-9 (> 40 kb in size) were identified.
Conclusions: Results obtained here indicate extensive LD within the *CYP19* region and thus addressing the suitability of the region for case-control association studies. In addition, findings in *CYP19* genomic region support the idea that the human genome is arranged into block-like patterns.

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NICASTRIN POLYMORPHISMS DO NOT MODIFY THE RISK FOR LATE-ONSET ALZHEIMER'S DISEASE IN A FINNISH CASE-CONTROL STUDY.

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Background: Nicastrin (NCSTN), a type 1 transmembrane glycoprotein, modulates presenilin proteins (PSENs) and Aβ production. The gene encoding NCSTN maps to 1q23, a region that has been associated with late-onset Alzheimer's disease (LOAD) in different genome screens. We evaluated association of NCSTN polymorphisms to the occurrence of AD in 133 cases and 189 matched controls ascertained in the eastern Finnish population.
Methods: Four SNPs in NCSTN were selected that in the Dutch population captured the majority of haplotypes (A-D, 98%): one coding (exon 6:c.636A>G Leu212) and three non-coding variants (intron 6:IVS6+18C>G, intron 10:IVS10-5C>G and intron 16:IVS16-119G>C). The SNPs were genotyped in patients and controls using pyrosequencing method.
Results: None of the 4 SNPs showed statistically significant differences in genotype frequencies in the total AD sample nor in the early-onset AD (EOAD) sample when compared to their controls. Also, no significant genotypic association was found when stratifying the study groups according to APOE E4 status. In the total AD sample without APOE4 alleles, we found a borderline significance (P=0.05) in overall four-loci haplotype distribution when compared with controls.
Conclusions: In general, our results confirmed that NCSTN has a minor role in LOAD as a genetic risk factor and a role for NCSTN in EOAD risk remains questionable in Finnish population.

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SELADIN-1 TRANSCRIPTION IS LINKED TO NEURONAL DEGENERATION IN ALZHEIMER'S DISEASE

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Background: Seladin-1 is a gene recently shown to be down-regulated in brain regions selectively degenerated in Alzheimer's disease (AD). Our aim was to estimate the magnitude and specificity of seladin-1 down-regulation in AD and to study possible interactions between amyloid accumulation and seladin-1 expression in transgenic AD mouse model. The effects of wild type and mutant presenilin-1 to seladin-1 expression were studied in an okadaic acid induced apoptosis model using mouse N2a neuroblastoma cells.
Methods: Transcription of seladin-1 was assed in human and mouse samples using semi-quantitative RT-PCR.
Results: Transcription of seladin-1 was selectively down-regulated in the brain areas affected in AD. Down-regulation in seladin-1 transcription was associated with hyperphosphorylated tau seen as linkage to immunohistochemically detected paired helical filament tau, neuritic plaques and neurofibrillary tangles. In contrast, no association was found between seladin-1 transcription and β-amyloid deposition when human or transgenic mouse samples were analyzed. Furthermore, the relative transcription of seladin-1 was found to fluctuate during aging in the transgenic mouse model of AD. Finally, seladin-1 transcription was found to be up-regulated in mouse N2a cells induced to undergo apoptosis with okadaic acid.
Conclusions: The results presented here indicate that seladin-1 transcription is selectively down-regulated in brain regions vulnerable to AD and this down-regulation is associated with the hyperphosphorylation of tau-protein.

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HIPPOCAMPAL INTERNEURONS AND POLYSIALYLATED NEURAL CELL ADHESION MOLECULE IN ALZHEIMER'S DISEASE

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Background. Previously we have reported that interneurons containing calretinin (CR), parvalbumin (PV) and calbindin-D28k (CB) are differentially vulnerable in the entorhinal cortex in Alzheimer's disease (AD). We have also found an increased number of polysialylated neural cell adhesion molecule (PSA-NCAM)-immunoreactive infragranular cells in the dentate gyrus of patients having AD. Here we examined CR, PV and CB interneurons and their possible content of PSA-NCAM in the hippocampus of AD and controls.
Methods. Hippocampal sections from 12 AD patients and 7 controls were immunostained for CR, PV and CB and double immunostained for CR, PV and CB with PSA-NCAM using fluorescent-labelled antibodies.
Results. Analyses revealed that CR-immunoreactive (ir) and CB-ir interneurons are largely unaffected, whereas PV positive cells are almost entirely lost in the dentate gyrus and CA1 area in AD. The colocalization percentage between different calcium-binding proteins and PSA-NCAM varied from 20 to 40. Interestingly, the percentage of CB interneurons coexpressing PSA-NCAM in the dentate gyrus in AD was significantly higher than in controls.
Conclusions. In AD, CR, PV and CB interneurons show similar differential vulnerability in the hippocampus as in the entorhinal cortex. Unexpectedly, certain population of each of these interneuron types expresses PSA-NCAM. This is different from that observed in the rat hippocampus, where only CR cells contain PSA-NCAM.

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IDENTIFICATION OF OXIDATIVELY MODIFIED PROTEINS IN AGED AND ALZHEIMER'S DISEASE BRAIN.

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Background: There is a large body of evidence implicating the importance of oxidative stress in the pathogenesis of both Alzheimer's disease (AD) and aging. Oxidised proteins need to be identified in order to understand the relationship between protein oxidation, protein turnover, protein aggregation, and neurodegeneration.
Methods: Frontal cortex tissue of AD patients (n=10) with histopathologically confirmed diagnosis and age-matched controls (n=9) were used in the present study. Oxidised cytosolic proteins were studied by using 2 D oxyblots. Selected proteins were separated by 2D electrophoresis and in-gel digested with trypsin for the mass spectrometric analysis.
Results: About 150 proteins and more than 100 oxidised proteins can be detected in both AD and control cases by 2D image analysis. The amount of protein-bound carbonyls was decreased for six and increased for one protein in AD. Furthermore, the degree of oxidation was calculated as the ratio of protein-bound carbonyls to the total amount of an individual protein. Three proteins showed a significant decrease in the degree of oxidation in AD. They were identified as two isoforms of cytosolic malate dehydrogenase and glutamate dehydrogenase.
Conclusions: In the present study, we have successfully applied 2D oxyblots and mass spectometry for identification of oxidatively modified proteins in aged and AD brain. Our results support the hypothesis of having alterations in the balance of oxidatively modified proteins in AD. Furthermore, we suggest that proteins that are less sensitive to oxidative stress may play a role in compensation of energy losses in AD by supporting alternative metabolic routes.

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REGIONAL DISTRIBUTION OF a-SYNUCLEIN PATHOLOGY IN UNIMPAIRED AGEING AND IN ALZHEIMER'S DISEASE

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Background: Amygdaloid complex (AC) was found to be highly vulnerable to α-synuclein (αS) pathology in both familial and sporadic Alzheimer's disease (AD) and recently, the incidental Lewy bodies (LBs) were identified primarily in the lower brainstem. Above challenges the traditional view that substantia nigra (SN) is the region primarily affected in the spectrum of LB disorders.
Design: We examined the immunoreactivity of αS in SN, nucleus basalis of Meynert (nbM) and AC in 850 subjects selected from our Brain Bank with or without concomitant AD pathology.
Results: αS positive structures were seen in at least one of the studied brain areas in 121 subjects (14%). The primarily affected region was SN (89%) followed by nbM with 73% and AC with 67%. Incidental LBs in the SN were seen in subjects with no αS pathology in the lower brainstem. Thirty-four percent of the 77 sporadic AD patients diagnosed using CERAD criteria had αS pathology. SN and AC in these AD patients were equally affected.
Conclusions: A single predilection or induction site that would define developing clinical symptoms i.e. dementia versus parkinsonism could not be identified in this study. The fact that the classical AD pathology and αS pathology often co-exist in the brain does not mean that one is caused by another.

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BEHAVIORAL EFFECTS OF SUBCHRONIC MEMANTINE TREATMENT IN APP/PS1 DOUBLE MUTANT MICE MODELING ALZHEIMER'S DISEASE

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Background: Memantine is a moderate affinity uncompetitive NMDA receptor antagonist. It has recently been approved in several European countries for clinical use in the treatment of Alzheimer's disease. However, its mechanism of action isn't fully understood, and no studies so far have assessed its effects on transgenic animal models of Alzheimer's disease.
Methods: Male C57BL/6J mice carrying mutated human APPswe and PS1(A246E) genes (A/P, n=45) and nontransgenic littermates (NT, n=36) were assigned for the memantine or placebo treatments at the age of 8 months. Memantine (30 mg/kg) was administered orally for two weeks in the drinking water before the behavioral testing began. Motor and exploratory activities were assessed in an automated activity monitor. Social behavior was assessed by the intruder-induced aggression test. Spatial working memory was tested by spontaneous alternation in the T-maze, and spatial long-term memory in the Morris water-maze.
Results: A/P mice were hypoactive and showed less exploratory rearing in the activity test than NT. Memantine had no effect on locomotor activity in either genotype. A/P mice had a shorter latency to attack the intruder than NT. Memantine didn't affect aggressive behavior. A/P mice were slower to complete the T-maze test and also alternated less than NT. Although memantine-treated mice spent more time exploring the maze, they achieved similar alternation scores as non-treated. A/P mice had longer escape latencies to both hidden and visible platforms in the water-maze, indicating impaired spatial learning. Memantine significantly improved the acquisition of the water-maze, but didn't affect swimming speed.
Conclusion: Memantine has beneficial effects on cognition.

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GALANTAMINE DEMONSTRATES GREATER EFFICACY THAN DONEPEZIL IN 52 WEEKS OF ACTIVE TREATMENT OF PATIENTS WITH ALZHEIMER'S DISEASE: AN INITIAL ANALYSIS

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Background: Galantamine and donepezil are approved for treatment of mild-to-moderate Alzheimer's disease (AD). Here, we compare the long-term efficacy of galantamine and donepezil in AD.
Methods: In a 52-week, multicenter trial, patients were randomized to galantamine 4 mg bid (escalated to 8 mg bid at Week 4; option to increase to 12 mg bid at Week 13) or donepezil 5 mg qd (option to increase to 10 mg qd at Week 4). The primary objective was to compare effects of galantamine and donepezil on functional abilities (BrADL), with additional comparisons on cognition (MMSE, ADAS-cog) and attention (CDR Battery).
Results: At study end, 80.4% of galantamine and 78.0% of donepezil patients remained on therapy. At Week 52, similar percentages of patients in each group demonstrated a positive response on the BrADL (GAL, 39.3% vs DON, 39.0%, p = 0.9679). More galantamine-treated patients demonstrated a favorable response on the ADAS-cog (GAL, 44.9%, DON, 31.7%, p = 0.0766) and MMSE (GAL, 55.2%, DON, 32.5%, p = 0.0036) at Week 52. All attention tasks (CDR) were improved with galantamine, with the most pronounced significant changes at Week 6.
Conclusions: Significant advantages were found with galantamine vs donepezil on cognition and attention; thus, it should be considered first-line treatment for AD patients.

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EFFECT OF CHOLINERGIC MEDICATION ON HISTOLOGICAL AND FUNCTIONAL OUTCOME IN YOUNG AND OLD RATS FOLLOWING CORTICAL PHOTOTHROMBOSIS

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Background. Functional recovery following stroke is highly variable depending on the location and size of the infarct, and medication the patient receives. The present study evaluated the effect of galanthamine, a selective competitive cholinesterase inhibitor on histological and functional outcome after experimental stroke in rats.
Methods. Young (5 month) and old (24 month) rats were treated with saline or galanthamine at a dose of 2.5 mg/kg (i.p., once a day). Drug treatment was started 4 days before cortical photothrombosis (Rose Bengal) and continued for 21 days thereafter. Sensorimotor recovery was assessed by beam-walking test and spatial learning by Morris water-maze 2 hours after drug administration over a 3-week follow-up period. Infarct volumes were measured from nitroblue tetrazolium -stained sections.
Results. Infarct volumes in the cortex were similar in ischemic controls and ischemic rats treated with galanthamine. Analysis of water-maze data did not reveal significant differences between sham-operated, ischemic controls and ischemic rats treated with galanthamine in escape latency, length or speed. In the beam-walking test, there was a transient impairment in forelimb and hindlimb function after cortical infarct both in young and old rats. Galanthamine treatment did not affect the sensorimotor recovery rate.
Conclusions. Galanthamine seemed to be safe with respect to the histological and functional outcome in rats subjected to cortical photothrombosis.

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REHABILITATION IN MILD ALZHEIMER'S DISEASE

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Background. Previous studies have suggested that early rehabilitation and extensive caregiver support may delay the institutionalization of Alzheimer patients. We have started a randomized controlled intervention study concerning the effects of rehabilitation in mild Alzheimer's disease (AD) in Northern Savo and Northern Karelia in Finland. The research project utilizes approaches based on medical science, nursing science and health economics.

Design. Recently diagnosed AD patients and primary caregivers (n=300 pairs) will be recruited and randomized into an intervention group (n=100 pairs) and a control group (n=200 pairs). The follow-up time will be five years. Different studies will be conducted within the research project. The nursing science subproject will study primary caregivers' life control using multiple assessments. Life control will be studied by qualitative methods to discover primary caregivers' subjective meanings. The study will also compare the changes in daily functioning, quality of life and health status between the intervention and control groups. Health economics research will provide information of the effectiveness of the treatments measured as QALYs. It will also provide computer modelling for assessing the health-related and economic outcomes of the intervention using institutionalization as a primary outcome.

Conclusions. This research project will give valuable information for the Finnish health care system about the effectiveness of early rehabilitation of Alzheimer patients and their primary caregivers. It will also be the first Finnish research that will widely studies patients' and their primary caregivers' life control and quality of life.