

# Satellite symposium, Monday, May 21th 2007

Doorwerth,

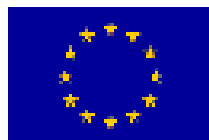
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Time: 13:00 – 16:30 h

## **Alzheimer's disease: new insights from animal models and molecular pathways, to be translated into human pathology**

9th Annual Genes, Brain and Behavior Meeting  
of the International Behavioural and Neural Genetics Society (IBANGS)  
Doorwerth, The Netherlands, 21-25 May 2007

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The International Behavioural and Neural Genetics Society



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**NEURAD**  
Neurodegeneration in Alzheimer's disease  
Marie Curie Ph.D. Graduate School

Chairs            Thomas Bayer, Saarland University, Germany  
Fred Van Leuven, Leuven University, Belgium

13.00:            Samir Kumar-Singh, Univ. Antwerp, Belgium

The mysteries of amyloid: fibrillar–nonfibrillar, intracellular–extracellular, A $\beta$ 40–42:  
where does it all lead to?

13.20:            Benoit Delatour, Univ. Paris-Sud, France

Behavioral phenotype of a new transgenic mouse model replicating cerebral amyloidosis and  
neuronal loss of Alzheimer's disease

13.40:            Kathy Keyvani, Univ. Münster, Germany

Alzheimer's disease: effects of environmental enrichment

14.00:            Thomas A. Bayer, Saarland Univ., Germany

Synaptic deficits correlate with neuron loss and changes in behaviour in the APP/PS1ki  
mouse model

Break

15.00:            Chris Janus, Mayo Clinic, Jacksonville, USA

Inducible tau mice

15.20:            Paul Lucassen, Univ. Amsterdam, the Netherlands

Increased LTP and learning but not neurogenesis in young tau-P301L mice

15.40: Katharina Schindowski, Univ. Lille, France

Alzheimer's disease-like imbalance of neurotrophic factors in a tau transgenic model

16.00: Fred Van Leuven, KULeuven, Belgium

Bigenic transgenic mice: amyloid and tau pathology are linked by GSK-3 $\beta$

General discussion

**Abstract of symposium proposal:** A model for a human disease is defined as an experimental preparation developed for the purpose of studying a condition in the same or different species. Typically, models are animal preparations that attempt to mimic a human condition. In developing and assessing an animal model, it is critical to consider and explicit purpose intended for the model. The intended purpose determines the criteria that the model must satisfy to establish its validity. In this context we will discuss different animal models for Alzheimer's disease (AD), which have been developed based on current knowledge of the neurotoxic cascade.

Pathological alterations in the brain of AD patients are characterized by loss of synapses and neurons in specific brain regions leading to its typical clinical symptoms, like memory impairment and change in personality. According to the  $\beta$ -amyloid hypothesis, A $\beta$  peptides liberated from its precursor APP is deposited in A $\beta$ -amyloid plaques, which trigger the pathology, however, plaques don't correlate with the clinical symptoms and loss of neuronal function. Therefore one needs to critically discuss the prevailing  $\beta$ -amyloid hypothesis. The focus of the symposium is to discuss novel ideas of the neurotoxic cascade: evidence for N-truncated A $\beta$  peptides, ratio of A $\beta$  40/42, intraneuronal aggregation, and role of Presenilin-1 on APP processing. A $\beta$ 40 might be even protective by perhaps sequestering the more toxic A $\beta$ 42 and facilitating its clearance. Alternatively, there may be a potentially detrimental effect of accumulating APP C99 fragments, which demands further study of the consequences of inhibition of  $\gamma$ -secretase activity. In addition, the complex functional relation of APP and PS1 to cognition and neuronal plasticity in adult and aging brain will be discussed in transgenic mouse models. On the basis of the  $\beta$ -amyloid pathology novel mouse models for AD have been developed. Some of them show a complex pattern of neuron loss, synaptic dysfunction, brain atrophy, behavioural deficits and AD-typical age-dependent pathology. We will discuss the effect of AD-typical pathology on behavioural phenotyping, its effects on exploratory activity, motor coordination, and working memory and other learning paradigms, and the influence of environmental on these measures.

While there may be no perfect animal models for AD, it is clear that each model has strengths and limitations that need to be recognized in order to use the model effectively. The process of developing and validating animal models for AD must work in concert with the process of identifying reliable measures of the human AD pathology and phenomenology.

## Details of speakers and abstracts

### Speaker 1: Samir Kumar-Singh

Neurodegenerative Brain Diseases Group, VIB8 Department of Molecular Genetics, Flanders Institute for Biotechnology, University of Antwerp, Universiteitsplein 1, B-2610 Antwerpen, Belgium

Email: [samir.kumarsingh@ua.ac.be](mailto:samir.kumarsingh@ua.ac.be)

### **Title: The mysteries of A $\beta$ amyloid: fibrillar–nonfibrillar, intracellular–extracellular, A $\beta$ 40–A $\beta$ 42: where does it all lead to?**

**Abstract:** While losing some of the panache A $\beta$  enjoyed in the Alzheimer's disease (AD) field in the past, it still maintains a dominant position in the disease cascade. This is because no other molecule is as intricately linked to the AD pathogenesis as A $\beta$ . Although this should not deter us from exploring alternative therapeutic possibilities, more efforts should also be put in understanding: (i) the precise steps in the aggregation of A $\beta$  as well as of A $\beta$  aggregation states that are neurotoxic; (ii) other pathological alterations that might be tightly linked to A $\beta$  alterations and in turn contribute to dementia, i.e., altered calcium homeostasis; (iii) the physiological function of A $\beta$  – A $\beta$ 42 versus A $\beta$ 40 (or A $\beta$ 39 etc.) and what does the A $\beta$ 40:A $\beta$ 42 ratio mean; (iv) the precise compartment intracellular A $\beta$  localizes to and if it is related to dementia as well as cellular alterations noticed in AD patients and in mouse AD models. I will discuss some of these issues in context of some current themes of research being performed in our group. Mostly, I will focus on two things. First, I will discuss some of the recent findings from our laboratory showing that all presenilin mutations studied significantly increased A $\beta$ 42:A $\beta$ 40 ratio *in vitro*, significantly decreased absolute levels of A $\beta$ 40 (with accumulation of APP C-terminal fragments), while only half of the mutations studied significantly increased A $\beta$ 42. Interestingly, not only an increase in the absolute levels of A $\beta$ 42 or A $\beta$ 42:A $\beta$ 40 ratio, but also a decrease in A $\beta$ 40 levels correlated with the age-of-onset of presenilin 1-linked familial AD. Secondly, I will discuss a novel mouse model expressing APP/Austrian mutation (T714I) – that was earlier shown to cause one of the highest A $\beta$ 40:A $\beta$ 42 ratio with a remarkable 80% drop in A $\beta$  – to deposit intraneuronal A $\beta$  and reduced brain volumes and fits well with the hypothesis that intraneuronal A $\beta$  could be toxic

**Speaker 2: Benoit Delatour**

Laboratoire de Neurobiologie de l'Apprentissage, de la Mémoire & de la Communication, NAMC, CNRS UMR 8620, Université Paris-Sud, 91405 Orsay Cedex, FRANCE

Email: benoit.delatour@ibaic.u-psud.fr

**Title: Behavioural phenotype of a new transgenic mouse model replicating cerebral amyloidosis and neuronal loss of Alzheimer's disease.**

**Abstract:** One major caveat of mice transgenic models of Alzheimer's disease (AD) is their failure to mimic all aspects of human neuropathology. Most of the models reproduce brain amyloidosis (A $\beta$  plaques and amyloid angiopathy). Neuron loss as observed in human disease is, to date, still lacking in most of the currently available mice models. Dramatic loss of hippocampal neurons and severe brain amyloidosis have recently been reported in mice overexpressing mutated APP transgene coupled to knocked-in PS1 mutations linked to familial AD. The aim of our work was to investigate the nature and chronology of behavioural anomalies these APPxPS1-Ki mice are prone to develop. Cohorts including APP/PS1-Ki mice, PS1-Ki cell loss- and amyloid-free mice and wild type mice were, at first, longitudinally phenotyped at 2, 4 and 6 months of age. A first cohort was examined in a battery of neurological tests allowing to measure motor and locomotor functions, sensory abilities and anxiety-related behaviours. Results indicated that, even at very young ages, APP/PS1-Ki showed hyperactivity. These deficits worsened with progressive aging. Cognitive functions were assessed in a second cohort of mice. While short-term retention of information appeared to be preserved in APP/PS1-Ki mice (spontaneous spatial alternation task), gradual deterioration of spatial reference memory, as evidenced in the water maze task, was observed in the same animals. Behavioural analysis of mice trained in other hippocampal-dependent tasks are currently in progress. Finally, to identify which brain lesions (neuron loss, amyloid load, intracellular accumulation of A $\beta$ ) are responsible for neurological / cognitive defects, correlative analysis between behavioural performance and morphological markers (neuropathology, biochemistry, brain imaging) will be presented.

**Speaker 3: Kathy Keyvani**

Institute of Neuropathology, University of Münster, Germany

Email: kathy.keyvani@ukmuenster.de

**Title: Alzheimer's disease: effects of environmental enrichment**

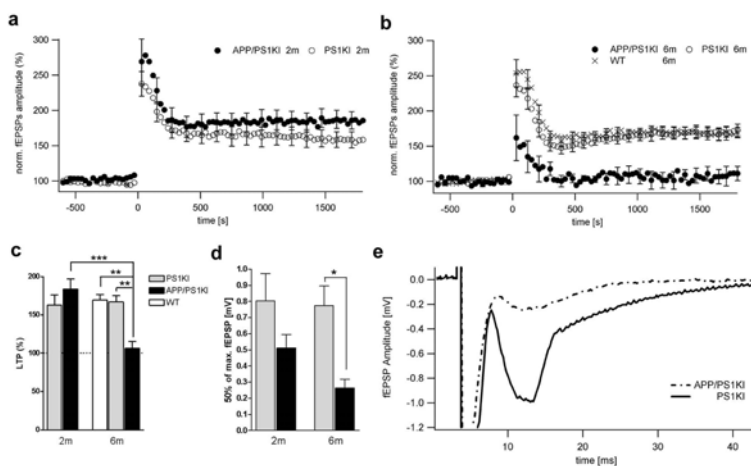
**Abstract:** Epidemiological studies suggest that the amount of time spent on intellectual and physical activities negatively correlates with the extent of cognitive decline and even risk of developing Alzheimer's disease (AD). Until recently, the most common explanation for this effect was that such lifestyle enhances the cognitive reserve and enables the patients to compensate the clinical expression of AD without affecting AD-related neuropathology. We and others have demonstrated that cognitive and physical stimulation in form of environmental enrichment in transgenic mice does interfere with AD neuropathology and results in significant reduction of cerebral A $\beta$  plaques and in a lower extent of amyloid angiopathy. This effect was independent from APP expression or processing and rather associated with reduced aggregation and enhanced clearance of A $\beta$ . The mechanism appears to be mediated by multiple pathways, in particular a reduced inflammatory response, enhanced microglial phagocytosis, proteasomal degradation, reduced cholesterol levels as well increased angiogenesis and differential regulation of A $\beta$  receptor / transporter molecules promoting A $\beta$  efflux across the blood brain barrier. This talk will give an overview on these findings indicating a hitherto unknown interplay between the environment and Alzheimer diseased brain.

**Speaker 4: Thomas A. Bayer<sup>1</sup>, Kailai Duan<sup>2</sup>, Henning Breyhan<sup>1</sup>, Jens Rettig<sup>2</sup>, Oliver Wirths<sup>1</sup>** Saarland University, Department of Psychiatry<sup>1</sup>, Institute of Physiology<sup>2</sup>, Homburg/Saar, Germany.

Email: thomas.bayer@uniklinik-saarland.de; new: tbayer@gwdg.de

**Title: Synaptic deficits correlate with neuron loss and changes in behaviour in the APP/PS1ki mouse model**

**Abstract:** The APP/PS1ki mouse model for AD was studied by different means. It expresses human mutant APP751 with the Swedish and London mutations co-expresses two FAD-linked knocked-in mutations (PS1M233T and PS1 L235P) in the murine PS1 gene (named APP/PS1KI mouse model). At the age of six months there is a robust pathology with changes in synaptic function, axonal degeneration, neuron loss due to massive intraneuronal accumulation of N-modified A $\beta$  X-42 peptides. Based on the observation that extracellular A $\beta$ -deposition in typical AD-plaques is not correlated with neuronal degeneration the  $\beta$ -amyloid hypothesis needs a revision with intraneuronal A $\beta$  as the major risk factor for AD.



**Fig. (a)** LTP recordings looked similar between 2-month-old APP/PS1KI mice (6 slices / 5 mice) and PS1KI mice ( $n = 5/4$ ), whereas LTP was almost abolished in 6-month-old APP/PS1KI mice (**b, c**) LTP was almost abolished ( $n = 4/3$ ), compared to WT ( $n = 8/5$ ) and PS1KI control mice ( $n = 10/4$ ). (**d**) At the age of 2 months the fEPSP recorded in slices from APP/PS1KI was similar to that from PS1KI mice, whereas at the age of 6 months the fEPSP recorded in APP/PS1KI mice was significantly reduced. (**e**) Example of half-maximal field excitatory post-synaptic potentiation (fEPSP) recordings (6 month-old). All data are given as means  $\pm$  s.e.m. \*\*\* $P < 0.001$ ; \*\* $P < 0.01$ ; \* $P < 0.05$ .

**Speaker 5: Chris Janus**

Mayo Clinic Jacksonville, Department of Neuroscience, 4500 San Pablo Rd., Birdsall 215, Jacksonville, FL 32224, USA

Email: Janus.Christopher@mayo.edu

**Title: Inducible tau mice**

**Abstract:** Protein tau, a microtubule-associated protein, is implicated in control and integrity of neuronal bidirectional transport. The abnormal accumulation of tau in neurofibrillary tangles occurs during normal aging and is a major pathological hallmark of neurodegenerative diseases known as tauopathies. Consequently, the elimination of toxic tau species through appropriate cellular degradation pathways may prevent or even reverse dementia. A recent study showed that the spatial memory deficits in a conditionally suppressible mouse model of tauopathy could be reversed when the production of tau was significantly lowered (Santa-Cruz, K. et al. 2005, *Science* 309, p.476-81). This mouse model, denoted rTg(tau<sub>P301L</sub>)4510, is characterized by age-related intraneuronal accumulation of insoluble tau and rapidly progressing neuronal death with coinciding cognitive decline. Since neurodegeneration progresses at different rates in individuals from the same age cohorts, we investigated the relationship between tau levels and memory loss in individual mice. Using cross-sectional design we tested Tg4510 mice in hippocampus-dependent spatial navigation task at ages of 3 months - corresponding to a pre-tangle formation stage, and at 5.5 months, when neurofibrillary tangles appear and a significant loss of neurons in the hippocampus was observed. The results show that the transgenic mice were significantly impaired in spatial memory as compared to non-transgenic littermates, and that this impairment was age progressing. The impairment in spatial navigation manifested itself by inability of the transgenic mice to use spatial strategies during learning acquisition and by poor spatial reference memory. The mice also showed distinctive, age-progressing tau pathology. While hyperphosphorylated tau species were detected in all mice from the 5.5 mo-old cohort, they were observed in only a few mice at the pre-tangle stage. The severity of deficits in the spatial memory of individual 5.5 mo old mice was significantly associated with increased levels of hyperphosphorylated tau species in the brain. The possible mechanism of the cognitive impairment in this mouse model of tauopathy will be discussed in the light of known cellular pathways for the degradation of tau.

**Speaker 6: PJ Lucassen<sup>1</sup>, K Boekhoorn<sup>1</sup> H Krugers<sup>1</sup>, D Terwel<sup>2</sup>, P Borghgraef<sup>2</sup>, M Joels<sup>1</sup> and F van Leuven<sup>2</sup>**

1. SILS - Center for Neuroscience, University of Amsterdam, The Netherlands.

2. Experimental Genetics Group. Dept. Human Genetics, K.U.Leuven, Leuven, Belgium

Email: lucassen@science.uva.nl

**Title: Increases in LTP and learning but not neurogenesis in young P301L tau mutant mice.**

**Abstract:** In dementia, the hippocampus is particularly affected and extensive tau pathology, aberrant cell-cycle protein expression and changes in neurogenesis have been reported. Neurogenesis has been implicated in hippocampal function and is a.o. stimulated by hippocampal injury. Interestingly, protein tau is implicated in tauopathies like frontotemporal dementia (FTD) but is also important for cell division and neuronal plasticity. Hyperphosphorylation of tau is crucial in the age-related formation of neurofibrillary tangles (NFTs) that correlate well with cognitive decline.

To address the consequences of the P301L mutation itself, we studied tau-P301L mice recapitulating the human phenotype, but at 2 months of age, prior to the onset of tau hyperphosphorylation and axonopathy. Unexpectedly, in these mice, increased long-term potentiation in the dentate gyrus was observed paralleled by an improved memory performance in a hippocampus dependent task. Neither tau phosphorylation, adult neurogenesis, nor other morphological parameters could account for this.

This implies that protein tau is involved in hippocampal function and that not the tau mutation per se, but rather the ensuing hyperphosphorylation must determine the cognitive decline in tauopathies.

Refs; Boekhoorn et al., J Neurosci 2006; Boekhoorn et al., Neurobiol. Dis. 2006.

PJL and KB are supported by the Internationale Stichting Alzheimer Onderzoek (ISAO).

**Speaker 7: Katharina Schindowski, Karim Belarbi, Alexis Bretteville, Séverine Bégard, Malika Hamdane, and Luc Buée**

INSERM, U815, Université Lille, 1 Place de Verdun, F-59045 Lille Cedex,  
France

Email: Katharina.Schindowski@lille.inserm.fr

**Title: Alzheimer's disease-like cortical and hippocampal imbalance of Brain-Derived-Neurotrophic-Factor and Nerve-Growth-Factor in a tau transgenic model**

**Abstract:** We have recently generated the THY-Tau22 mouse model, expressing human 4-repeat tau mutated at sites G272V and P301S under a thy1-promotor that shows Alzheimer's disease (AD)-like tau pathology, neurodegeneration, deficits in synaptic transmission and impaired memory. Here we report decreased levels of brain-derived-neurotrophic-factor (BDNF) and increased levels of nerve-growth-factor (NGF) in the hippocampus and cortex very similar to what is reported from AD post mortem brain. BDNF mRNA and protein levels are reduced by 6 months in tau transgenic animals. Interestingly, the loss of BDNF correlates negatively with tau abnormally phosphorylated at Thr212/Ser214 (AT100). In the entorhinal cortex, CA3, DG and to a lesser extent in CA1, we observed neurons that accumulate NGF-immunoreactivity. Interestingly, some of these neurons colocalize accumulated NGF and AT100, but not AT8 (tau phosphorylated at Ser202/Thr205) indicating a crucial role of abnormal phosphorylated tau in this process. Immunoblotting revealed that the levels of pro-NGF were significantly increased in THY-Tau22. However, in contrast to data from AD, we found a significantly increased number of DG neurons expressing NGF mRNA. This is the first mouse model showing an AD-like imbalance of BDNF and NGF in cortex and hippocampus. Since there are growing evidences for the relevance of neurotrophic factor distribution in the pathogenesis of AD, this model is a useful tool to investigate the underlying mechanisms.

**Speaker 8: Fred Van Leuven**

Experimental Genetics Group - LEGT\_EGG, Dept. Human Genetics, K.U.Leuven - Campus Gasthuisberg ON1-06.602, B-3000 Leuven, Belgium

E-mail: fredvl@med.kuleuven.be

**Title: Bigenic transgenic mice: amyloid and tau pathology are linked by GSK-3 $\beta$** **Abstract:**

The exact recapitulation of pathological processes in brain of AD patients remains a major target for experimental biologists. To this end, we have generated single and bigenic transgenic mice that develop pathological hallmarks of AD. Our APP-V717I transgenic mice progressively present amyloid pathology, characterized by intracellular amyloid, diffuse and senile plaques, vascular deposits, all progressively worsening with ageing. This "late" pathology is preceded by "early" defects in cognition and in hippocampal LTP, which was not rescued by neuron-specific inactivation of Presenilin1 (Moechars et al, 1999; Van Dorpe et al, 2000; Dewachter et al, 2002). Our Tau-P301L mice display morbid and moribund tauopathy with intracellular tau-filaments, resulting in mortality before age 1 year (Terwel et al, 2005) but, surprisingly, preceded by improved cognition at young age (Boekhoorn et al, 2006). Ageing APP-V717I x Tau-P301L bigenic mice (13-18 months) have combined AD-like pathology in hippocampus and cortex ("plaques and tangles"), with more extensive amyloid pathology than the parent APP-V717I mice, and with dramatic enhanced forebrain tauopathy, particularly in hippocampus CA1 (Muyllaert et al, 2006). Remarkably, the Tau-P301L mice die before age 1 year, while the APP-V717I x Tau-P301L bigenic mice survive longer, which is tightly correlated to the alleviation of brainstem tauopathy, which is much less prominent than in the parental Tau-P301L mice. Even more remarkable, also Tau-P301L x GSK-3 $\beta$  bigenic mice have a normalized lifespan relative to Tau-P301L mice, again correlating with strongly reduced brainstem tauopathy. Moreover, the -P301L x GSK-3 $\beta$  bigenic mice are characterized by a dramatic forebrain tauopathy with "tangles in almost every neuron". The combined data corroborate the conclusions that (i) neither amyloid nor neurofibrillary tangles are toxic per se, (ii) GSK-3 $\beta$  is a - or the - missing link between amyloid and tau-pathology, (iii) but with important brain-regional differences in its actions.