sensitive cell specific proteome analysis by LCM/MS, could be of general use for studies in health and disease.

**S3-03-02 INTRACELLULAR Aß AND NEURONAL DEATH**
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**Background:** Alzheimer’s disease (AD) is characterized by accumulation of extracellular amyloid plaques and the presence of intracellular neurofibrillary tangles. There is increasing evidence that Aß peptides accumulate also inside neurons, in addition to the well-known parenchymal extracellular amyloid deposition and it has been hypothesized that this represents one of the earliest events triggering the neurodegenerative cascade. Transgenic mice have been proven to be valuable systems to model early intraneuronal Aß accumulation and the resulting pathological consequences. 

**Methods:** Transgenic AD mouse models (APP/PS1K1, 5XFAD, TBA2) were used to investigate if significant increases in the amount of diverse Aß species within neurons coincided with neuron loss in the respective brain regions. The presence of extracellular amyloid plaques and intraneuronal Aß accumulations was assessed by immunohistochemical stainings using antibodies against a variety of Aß peptides. Design-based stereology was used to quantify neuron numbers in transgenic mouse brains harbouring either only extracellular, or extra- and intracellular Aß pathology. 

**Results:** No significant neuron loss was detected in brain regions where only extracellular plaque deposition has been detected. The presence of intracellular Aß, however, coincided with significantly reduced neuron numbers in diverse brain regions like frontal cortex, CA1 or cholinergic brain stem nuclei in APP/PS1K1 mice or cortical layer 5 in 5XFAD mice. In addition, the intracellular expression of truncated Aß3-42 resulted in massive neurological impairments and abundant neuronal loss in TBA2 mice. These results lead to the assumption that extracellular Aß pathology does not significantly contribute to neuron death. 

**Conclusions:** In summary, these observations support a pivotal role of intraneuronal Aß as a major trigger in AD-typical neurodegeneration and neuron loss, whereas plaques might represent ‘wastebins’ for toxic peptides. The link to Tau is presently not fully understood.

**S3-03-03 FUNCTIONAL CORRELATIONS OF INTRACELLULAR Aß PEPTIDE IN TRANSGENIC MOUSE MODELS**
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**Background:** The amyloid cascade hypothesis has been continuously challenged since its initial formulation; it has evolved to take into account new data and speculations related to Alzheimer’s disease (AD) physiopathology. In particular the identification of an intraneuronal pool of A-beta peptides has favored new conjectures and might motivate additional revisions of the cascade hypothesis. 

**Methods:** To better address the functional impact of intracellular A-beta accumulation in AD, we made use of APPxPS1-Ki mice (overexpressing Swedish and London APP mutations, and PS1 M233T and L235P mutations) that develop early aggressive brain amyloidosis plus intracellular A-beta accumulation. The mice were studied between 2 and 4 months of age, a short time window corresponding to the initiation of brain lesions in this model. Cognitive evaluation and postmortem biochemical/morphological analysis were performed on the studied mice to establish possible correlations between symptoms and histopathological markers.

**Results:** First signs of cognitive impairments were detected at the age of 3 months and worsened with progressive aging. Between 2 and 4 months, extra-cellular amyloid loads (volume occupancy of plaques) increased but were uncoupled to the onset and aggravation of learning & memory deficits. At 2 month of age A-beta was hardly detectable by ELISA dosages in intracellular fractions but neurons accumulating A-beta material were evidenced by immunohistochemistry on brain sections. Both biochemical analyses and histological quantifications underlined a steady and linear increase of intracellular A-beta between 2 and 4 months of age. Correlative analysis in the 4-months old mice indicated that mice harboring the highest intracellular amyloid loads were the animals with the lowest learning profficiencies. An analysis of behaviorally-evoked immediate early genes (IEGs) underlined a decrement of neuronal activation in APPxPS1-Ki mice. Complementary analysis suggested a repressive role of intracellular A-beta on IEGs products known to play a crucial role in neural plasticity required for learning and memory functions. 

**Conclusions:** In conclusion these data strengthen the contention that amorphous extracellular amyloid deposition (plaques) are not directly coupled to impaired brain functioning. On the other side we provided experimental findings to support that intraneuronal accumulation is a key element in the initiation and progression of cognitive symptoms in AD.

**S3-03-04 ALTERNATIVE SPlicing TO AND ETrACellular TAU: THE INITIAl STEPS OF TAu PATHOLOGY PROGRESSION IN TAuOPATHIES**
Fei Liu, New York State Institute for Basic Research in Developmental Disabilities, Staten Island, N.Y., United States.

**Background:** Aggregation of hyperphosphorylated tau into neurofibrillary tangles in the brain is a hallmark lesion of Alzheimer’s disease (AD) and related tauopathies. Normal adult human brain expresses approximately equal level of 3R (three microtubule-binding repeats)-tau and 4R-tau generated from the alternative splicing of tau exon 10. 3R-tau and 4R-tau have different ability to stimulate microtubule assembly, to be phosphorylated by kinases and to aggregate. Dysregulation of the alternative splicing of tau exon 10 causes neurofibrillary degeneration. Therefore, human brain requires balanced expression of tau isoforms to maintain its function. Dysregulation of intracellular calcium signaling has been implicated in the pathogenesis of AD. As the major Ca2+-regulated protease in the central nervous system, calpain I is over-activated in AD brain. The goal of this study is to elucidate the molecular mechanism involved in tau pathogenesis from alternative splicing to phosphorylation of tau. 

**Methods:** By using immuno-blot, in vitro proteolysis and enzyme activity assay, we determined the levels of 3R-tau, 4R-tau, phosphorylated tau, Dysk1A (dual-specificity tyrosine phosphorylation-regulated kinase 1A), PKA (protein kinase A) and GSK-3beta (glycogen synthase kinase 3beta) in AD and control brain tissues, investigated the effect of proteolysis by calpain I on their activities and analyzed the relationship of their truncation with calpain I activation and 3R-4R-tau ratio and tau phosphorylation. 

**Results:** We found that truncations of PKA, Dysk1A, and GSK-3beta were correlated to the activation of calpain I. Calpain I cleaved Dysk1A, and GSK-3beta, resulting in a reduction of PKA activity and an elevation of Dysk1A and GSK-3beta activities. Reduction of PKA and elevation of Dysk1A and GSK-3beta promoted 3R-tau expression by modulating the function of ASF and SC35 on tau exon 10 inclusion. Increased Dysk1A activity phosphorylated tau and primed tau to be better substrate by GSK-3beta. The ratio of 3R-tau/4R-tau and tau phosphorylation correlated with calpain I activation and the truncation of PKA, Dysk1A, and GSK-3beta. 

**Conclusions:** Our results suggest that proteolytic cleavages of PKA, Dysk1A, and GSK-3beta by over-activated calpain I lead to increased 3R-tau/4R-tau ratio and abnormal tau hyperphosphorylation. These findings provide a mechanistic linkage between dysregulation of calcium homeostasis and neurofibrillary degeneration in AD and other tauopathies.

**S3-03-05 TRANSMISSION AND SPREADING OF TAuOPATHY IN TRANSGENIC MOUSE BRAIN**
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**Background:** Hyperphosphorylated tau makes up the filamentous intracellular inclusions of several neurodegenerative diseases, including Alzheimer’s disease. In the disease process neuronal tau inclusions first appear in transentorhinal cortex, from where they appear to spread to hippocampal formation and neocortex. Cognitive impairment becomes manifest when inclusions reach the hippocampus, with abundant neocortical tau inclusions and extracellular β-amyloid deposits being the defining pathological hallmarks of Alzheimer’s disease. Abundant tau inclusions, in the absence of β-amyloid deposits, define Pick’s disease, progressive supranuclear palsy,
soluble high-molecular weight oligomers may exacerbate pre-existing tau processes develop independently of one another. One can speculate that high levels of brils, and fibrils were observed in AD cases than in non-demented cases with and without Aß-pathology, more high-molecular weight oligomers correlates with one another as well as with the degree of pathology (NFT stage) was determined according to Braak and Braak 1991 (Acta Neuropathol., 82; 239-259), that of SP distribution (Aß phase) according to Thal et al. 2002 (Neurology, 58; 1791-1800). To detect soluble Aß-aggregates soluble fractions of brain lysates were analyzed with BN-PAGE, SDS-PAGE, and immunoprecipitation with subsequent western blot analysis. Results: In the human brain, the first AT8-immunoreactive pretangles occur in the locus coeruleus in very young individuals (Braak & Del Tredici, 2011, Acta Neuropathol. 121:171-181), then in the upper raphé nuclei and the transentorhinal cortex (allocortex), whereas Aß-deposits are first seen in the neocortex. In most cases, NFT-pathology precedes Aß-deposition. Both Aß-deposition and NFT-pathology progress in hierarchical sequences into further brain regions as described by the NFT stages and the Aß phases. Although the anatomical progression pathways differ, the time course of both pathologies correlates with one another as well as with the degree of dementia. Soluble Aß-oligomers, protofibrils, and fibrils have been discussed as being causative for the disease. Upon comparing AD cases with non-demented cases with and without Aß-pathology, more high-molecular weight Aß-aggregates were found in the native soluble fraction of AD cases than in that of non-demented cases with Aß-plaque pathology. Using immunoprecipitation, higher levels of SDS-denaturable Aß-oligomers, protofibrils, and fibrils were observed in AD cases than in non-demented individuals. In cases without Aß-plaques that exhibited initial tau pathology (NFT stages I or II) we did not detect measurable levels of Aß aggregates.

Conclusions: Thus, initial Aß pathology as well as initial tau pathology may develop independently of one another. One can speculate that high levels of soluble high-molecular weight oligomers may exacerbate pre-existing tau pathology by allowing it to cross the threshold for developing AD.

TUESDAY, JULY 19, 2011
FEATURED RESEARCH SESSIONS F3-01
PSYCHOSOCIAL INTERVENTIONS FOR INDIVIDUALS WITH DEMENTIA AND THEIR CAREGIVERS

Chairs: Simon Forstmeier, University of Zurich, Zurich, Switzerland
May Mittelman, NYU Langone Medical Center, New York, N.Y., United States

F3-01-01 COGNITIVE-BEHAVIORAL TREATMENT FOR MILD ALZHEIMER’S PATIENTS AND THEIR CAREGIVERS (CBTAC): A CASE STUDY OF A COMPREHENSIVE TREATMENT APPROACH
Simon Forstmeier, University of Zurich, Zurich, Switzerland.

Background: About 90% of all mild Alzheimer’s dementia (AD) cases experience neuropsychiatric symptoms, most frequently depression, anxiety, and irritability. Although some research has supported the effectiveness of specific psychotherapeutic approaches for mild AD, there are only few attempts to evaluate a comprehensive, CBT-based, multi-component treatment programme. The CBTAC study is a randomized controlled trial (RCT) that evaluates the effect of such a psychotherapy programme on the health of patients with mild AD and their caregivers. Methods: Participants are recruited via geriatric and memory clinics and general practitioners. The patients and their caregiver will be randomized to either the CBT-based intervention or to the control condition that receives treatment as usual (TAU). The CBT-based programme consists of 20 weekly sessions, including eight modules: diagnosis and goal setting; psychoeducation; engagement in pleasant activities; cognitive restructuring; live review; behavior management; interventions for the caregiver; and couples counselling. TAU is defined as receiving at least three out of six interventions: psychoeducation; appropriate medical treatment; social counselling; memory training; patients’ self-help group; caregivers’ self-help group. Before and after the treatment phase, participants in both conditions will be assessed. Follow-ups will take place at 3, 6, and 12 months post-treatment. A single case study will be presented to illustrate the comprehensive treatment approach. A multiple-baseline design was applied. Primary outcome measure is depression, assessed by the Geriatric Depression Scale and the Cornell Scale for Depression in Dementia. Further instruments assess other neuropsychiatric, functional and cognitive symptoms as well as coping strategies of the patients, and depression, anxiety, anger, general health and coping of the caregiver. Results: Data collection is currently underway. The results of the single case study to provide evidence of benefit of the psychotherapeutic intervention will be presented. Additionally, preliminary results of group level analyses will be presented. Conclusions: Findings will be discussed with respect to their conceptual, empirical, and clinical implications. There is an urgent need to establish a comprehensive psychotherapy treatment programme that does not only focus on a single strategy, but incorporates a multi-component treatment programme. If emotional health of cognitive impaired individuals could be effectively treated, the health care costs could be reduced significantly.