Cognition and brain plasticity in 5XFAD mouse model of Alzheimer's disease

Katrin Sonn (PhD student), Külli Jaako (PhD), Rajeev Kumar Jain (PhD), Alexander Zharkovsky (professor)

Department of Pharmacology, University of Tartu, Ravila 19, 50411 Estonia

Background

Alzheimer's disease (AD) is a neurodegenerative disease characterized by progressive deterioration of cognitive functions. Main histopathological findings in postmortem brains of AD patients include beta-amyloid (Aβ) - containing extracellular plaques and intracellular neurofibrillary tangles consisting of hyperphosphorylated Tau protein. Microglial activation and clustering around amyloid plaques, viewed as chronic inflammation, is a part of AD pathology [1]. Earliest signs of AD may be caused by various Aβ isoforms which disrupt synaptic function. Reexpression of developmentally regulated molecules, such as the polysialylated neural cell adhesion molecule (PSA-NCAM), which allows structural changes to occur in the brain, is viewed as the brain's attempt to restore its lost connections [2]. 5XFAD mice express 5 mutations characteristic of familial AD, being a model of rapid brain amyloid accumulation [3].

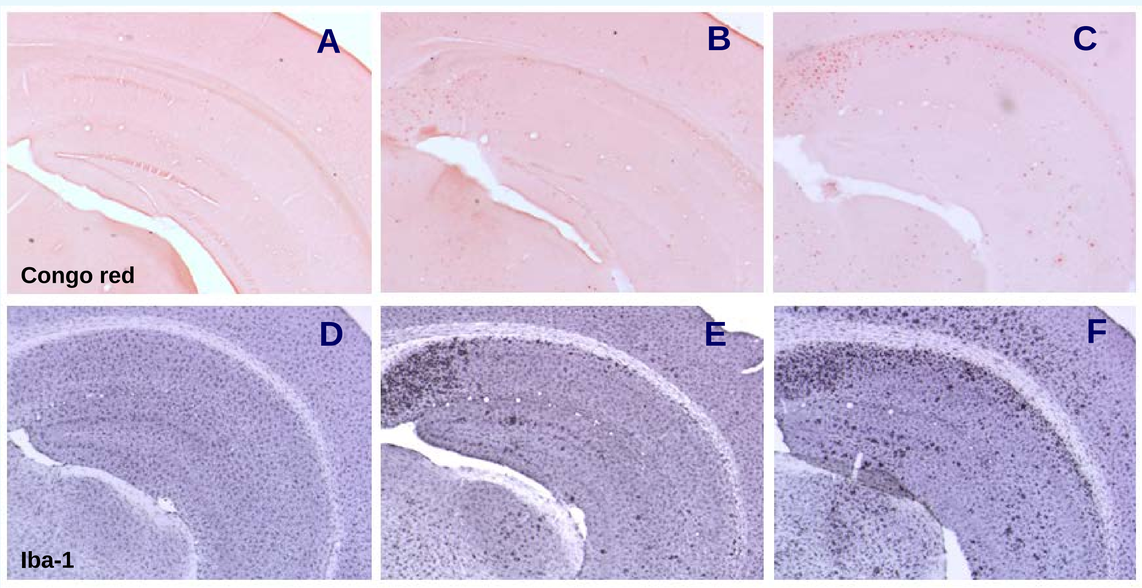

Figure 1. Congo red (marker for Aβ) and Iba-1(marker for activated microglia) stained brain sections (hippocampal region) of wild-type (**A, D**) and 5XFAD mice of 2 (**B, E**) and 6 months of age (**C, F**). Magnification 40 x.

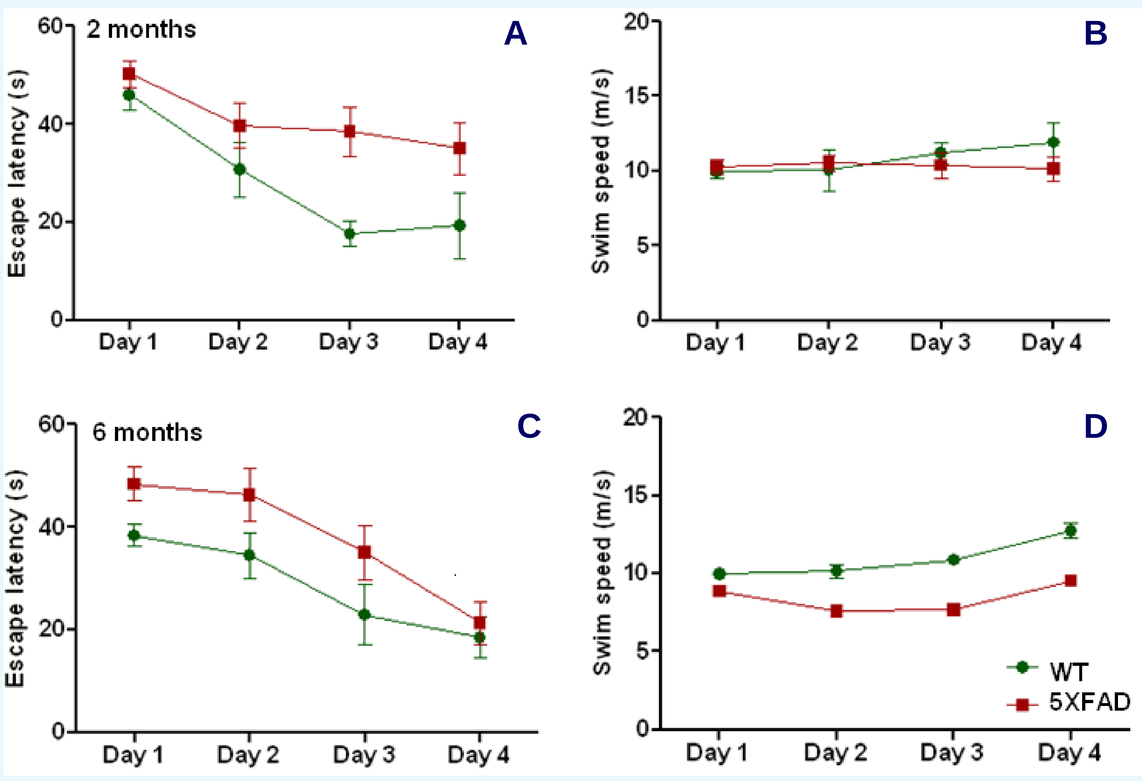

Figure 2. Escape latency (cut-off time 60 s) to find the hidden platform (**A, C**) and swim velocity (**B, D**) of 2 and 6 months old 5XFAD and wild-type mice in the Morris water maze task (MWM). Escape latencies and velocities on days 1-4 represent the average of two trials. Vision was assessed on day 5 by making the platform visible and measuring escape latency (not shown). Memory was assessed by removing the platform from the pool on day 6 and measuring percent of time (%) spent in the quadrant of the pool where the platform had been previously (not shown). Two-way ANOVA was used to compare data from days1-4; t-test was used to compare data from day 5 and 6; n=7-8 per group.

Aims

Our aim was to study whether the progression of hippocampal amyloidosis and microgliosis correlates with cognitive decline and changes in the levels of brain plasticity marker PSA-NCAM in 5XFAD mice.

Methods

5XFAD mice (C57/B6xSJL background) and wild-type littermates of 2 and 6 months were used. Experiments were performed according to European Union legislation by persons holding an appropriate license. Morris water maze task was performed as described previously [4]. Mouse brain sections were processed immunohistochemically and with Congo red stain, images were acquired with an Olympus BX-51 microscope and analyzed using ImageJ. The number of PSA-NCAM-positive cells was counted as in [5], levels of NCAM and PSA-NCAM were confirmed by immunoblot.

Results

Two months old 5XFAD mice had a significantly larger hippocampal Iba-1 positive area fraction than wild-type mice (5.45 ± 1.13 vs 2.36 ± 0.57%, *p=0.041), the difference had increased by 6 months of age (15.67 ± 2.61 vs 4.89 ± 0.66 %, **p=0.0052). Congo-red-positive hippocampal area fraction in 5XFAD mice was significantly larger at 6 than at 2 months of age (0.14 ± 0.01 vs 0.05 ± 0.01%, ***p<0.0001). Congo red-positive hippocampal Aβ plaque diameter in 5XFAD mice was significantly increased by 6 months compared to 2 months of age (42.20 ± 1.30 vs 31.26 ± 2.65 μm,**p=0.006). No Congo red-positive plaques were found in the brains of wild-type mice. Two months old 5XFAD mice learned slower in the MWM than wild-type mice (**Fig.2A**), (a significant effect of genotype ($F_{1,52}$=14.5, ***p=0.0004) and day ($F_{1,52}$=8.56, **p=0.001), but no interaction). 5XFAD and wild-type mice had no significant differences in swimming velocity, visual function or memory at 2 months of age. At 6 months of age, there was no difference in learning, memory or visual function between 5XFAD and wild-type mice. However, swimming velocity of 5XFAD mice was significantly lower compared to wild-type mice (**Fig. 2D**), with a significant effect of genotype ($F_{1,56}$=107.44, ***p< 0.0001), day ($F_{3,56}$=17.27, ***p< 0.0001) and an interaction ($F_{3,56}$=4.10, *p=0.011).

The number of PSA-NCAM-positive cells was significantly higher in 2, but not in 6 months old 5XFAD mice (**Fig. 3A**). By 6 months of age, the number of PSA-NCAM-positive cells had decreased significantly in 5XFAD and wild-type mice (**Fig. 3B**). Immunoblotting showed similar hippocampal levels of NCAM (**Fig.4A,B**), significantly higher levels of PSA-NCAM in 6 months old and a trend for increase in 2 months old 5XFAD mice (**Fig.4C,D**)

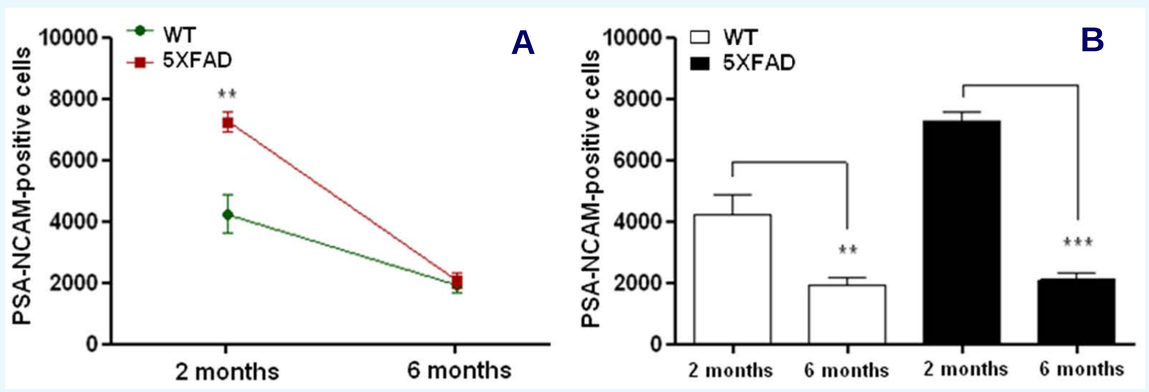

Figure 3. PSA-NCAM-positive cell number in the hippocampal subgranular zone of 2 and 6 month old 5XFAD and wild-type mice. **p<0.01,***p<0.001, t-test.

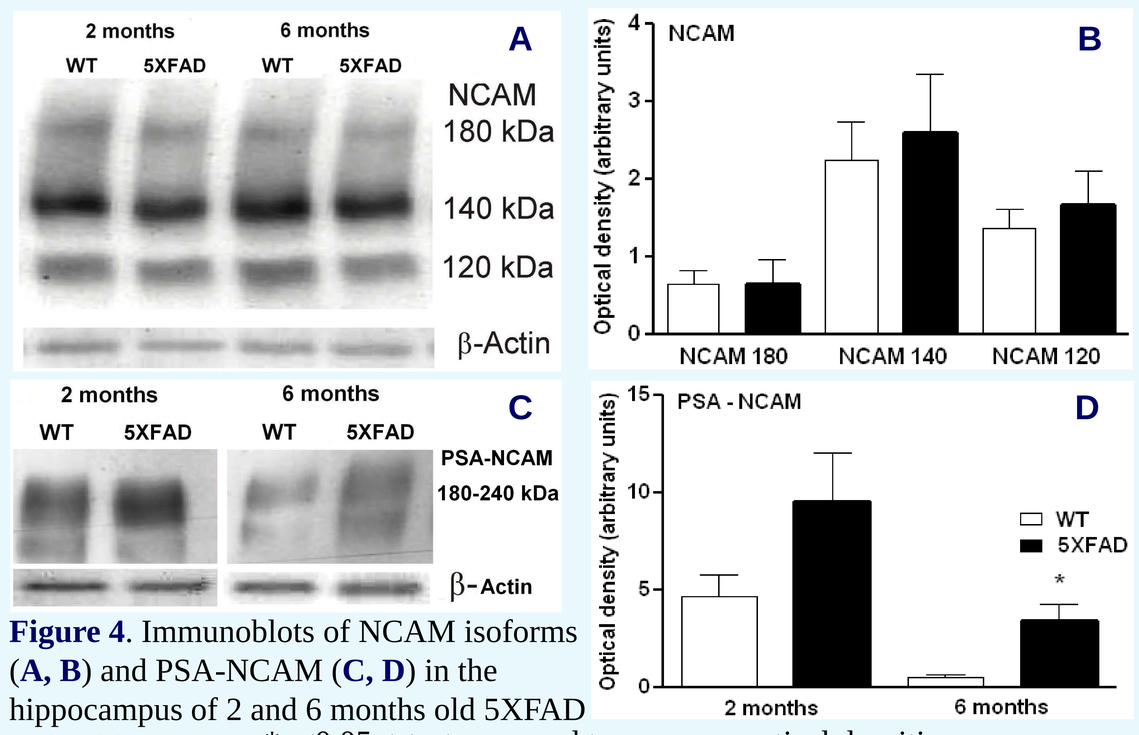

Figure 4. Immunoblots of NCAM isoforms (**A, B**) and PSA-NCAM (**C, D**) in the hippocampus of 2 and 6 months old 5XFAD and wild-type mice.*p<0.05, t-test was used to compare optical densities.

Discussion

The reason for not observing progressive cognitive impairment in 5XFAD mice despite a progression in hippocampal amyloidosis and microgliosis is unclear, suggestsing the existence of a compensatory mechanism. An increase in PSA-NCAM immunoreactivity in human AD brain has been reported [2] and it has been suggested that a high degree of structural plasticity might predispose neurons to tangle formation [6]. Higher level of PSA-NCAM of younger 5XFAD mice suggests that higher hippocampal plasticity might be a factor in AD pathogenesis. Increased PSA-NCAM expression might be caused by increased hippocampal neurogenesis and also by higher activity of enzymes linking PSA to NCAM (polysialyltransferases). The phenotype of PSA-NCAM-expressing cells is unclear as not only progenitors but also dedifferentiated mature cells can express PSA-NCAM .

1. Tahara K, Kim HD, Jin JJ, Maxwell JA, Li L, Fukuchi K. (2006) Role of toll-like receptor signalling in Abeta uptake and clearance. *Brain* 129, 3006-3019
2. Mikkonen M, Soininen H, Tapiola T, Alafuzoff I, Miettinen R. (1999) Hippocampal plasticity in Alzheimer's disease: changes in highly polysialylated NCAM immunoreactivity in the hippocampal formation. *Eur J Neurosci* 11:1754-1764
3. Oakley H, Cole SL, Logan S, Maus E, Shao P, Craft J, Guillozet-Bongaarts A, Ohno M, Disterhoft J, Van Eldik L, Berry R, Vassar R. (2006) Intraneuronal beta-amyloid aggregates, neurodegeneration, and neuron loss in transgenic mice with five familial Alzheimer's disease mutations: potential factors in amyloid plaque formation. *J Neurosci* 26, 10129-10140
4. Morris R. (1984) Developments of a water-maze procedure for studying spatial learning in the rat. *J Neurosci Methods* 11:47–60
5. Heidmets LT, Zharkovsky T, Jurgenson M, Jaako-Movits K, Zharkovsky A. (2005) Early post-natal, low-level lead exposure increases the number of PSA-NCAM expressing cells in the dentate gyrus of adult rat hippocampus. *NeuroToxicology* 27:39–43
6. Arendt T, Brückner MK, Gertz HJ, Marcova L. (1998). Cortical distribution of neurofibrillary tangles in Alzheimer's disease matches the pattern of neurons that retain their capacity of plastic remodelling in the adult brain. *Neuroscience* 83:991-1002

This poster is supported by European Social Fund and University of Tartu (Faculty of Medicine)