5XFAD mouse model of Alzheimer's disease: a dissociation between brain pathology and behavioural phenotype

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Background

Alzheimer's disease (AD) is a neurodegenerative disease and the most common cause of dementia worldwide. AD brain shows extraneuronal plaques consisting of β-amyloid (Aβ), intraneuronal aggregates of hyperphosphorylated Tau and neurofilament proteins, microglial activation as well as neuronal degeneration. Amyloid cascade hypothesis states that accumulation of Aβ in the brain is the primary pathogenetic step in AD.

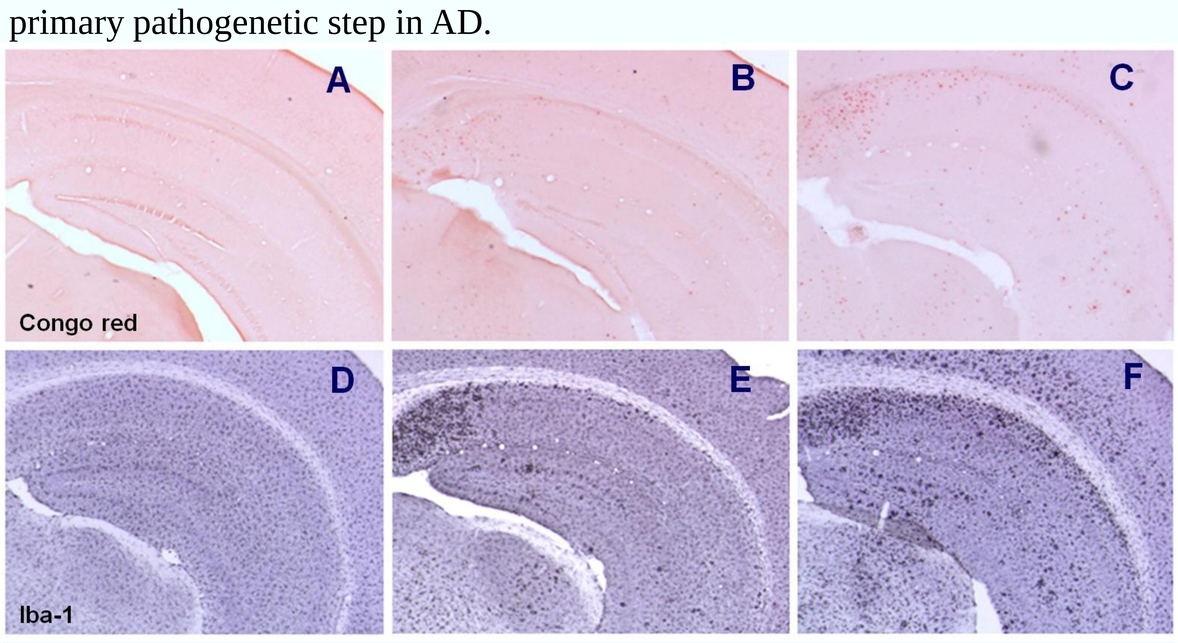

Figure 1. Light microscope images of Congo red (marker for Aβ) and Iba-1(marker for activated microglia) positive staining on brain sections (hippocampus) of wild-type (**A, D**) and 5XFAD mice of 2 (**B, E**) and 6 months of age (**C, F**). Magn. 20 x.

Aims and methods

5XFAD mice co-express 5 mutations of familial AD, being a model of rapid brain amyloidosis [**1**]. Our aim was to characterize this relatively novel model of AD by dynamically studying behavioural phenomena and brain findings of 5XFAD mice, to gain insight into AD pathology and to possibly identify novel drug targets. All experiments were done in accordance with EU legislation. 5XFAD mice and WT littermates (C57/B6xSJL background) of at 2 and 6 months of age were used.

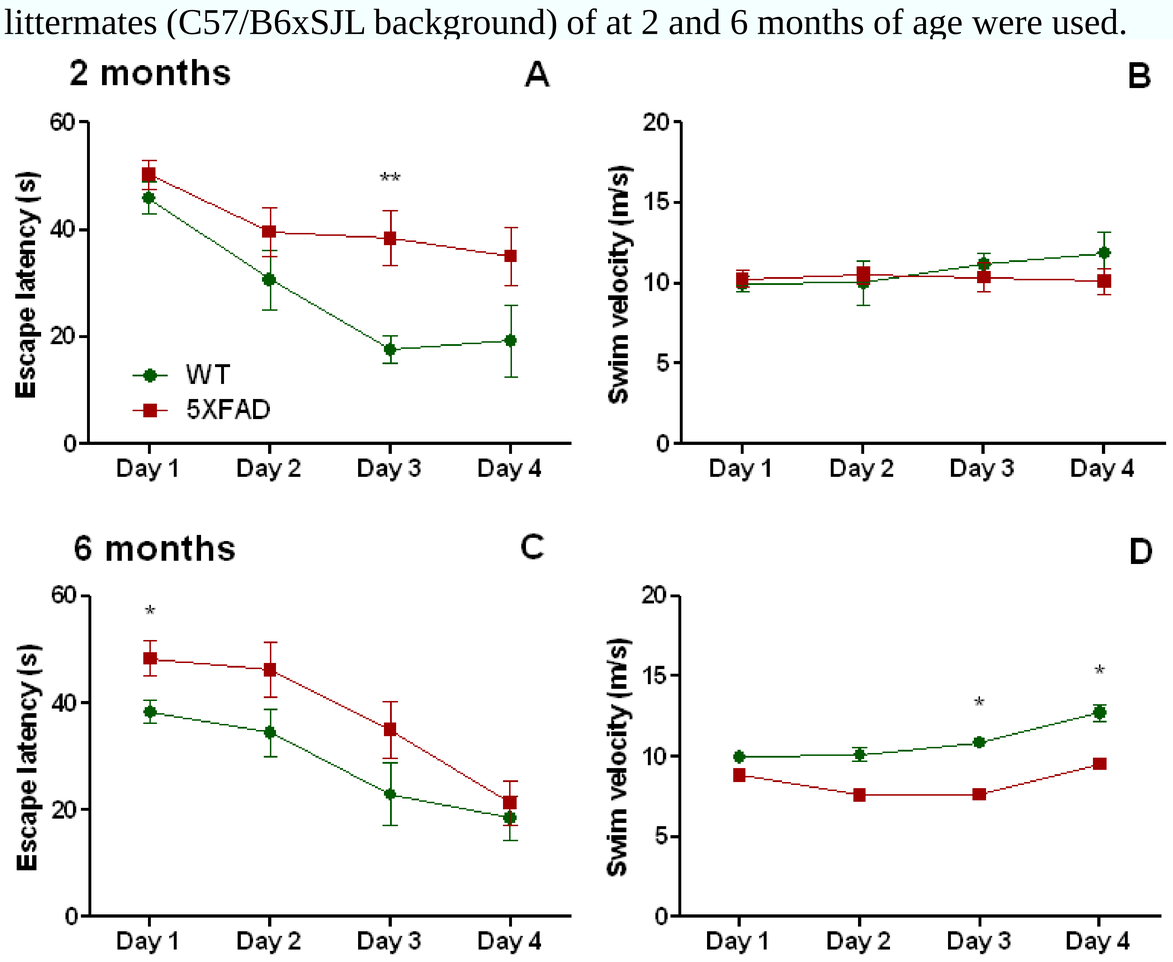

Figure 2. Escape latency to find the hidden platform (**A, C**) and swim velocity (**B, D**) of 2 and 6 months old 5XFAD and WT mice in the Morris water maze task (MWM). Escape latencies and velocities on days 1-4 represent the average of two trials. Vision was assessed on day 5 by making the platform 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 where the platform had previously been (not shown). *p<0.05,**p<0.01t-test; n=7-10.

Results

Congo red positive area % in the hippocampus of 5XFAD mice had grown by 6 months vs 2 months (0.14 0.01 vs 0.05 0.01%, ***p<0.0001). Aβ plaque size in the hippocampus of 5XFAD mice had grown by 6 months vs 2 months (42.20 1.30 vs 31.26 2.65 μm,**p=0.006). Iba-1 positive area % in the hippocampus of 5XFAD mice was larger vs WT mice at both 2 (5.45 1.13 vs 2.36 0.57 %, *p= 0.041) and 6 months (15.67 2.61 vs 4.89 0.66 %, **p=0.0052), **Fig. 1.**

In the MWM, 5XFAD mice in both age groups showed slightly impaired learning on some training days (**Fig. 2**) and no impairment in memory or visual function. The number of hippocampal PSA-NCAM positive cells was significantly higher in 2, but not 6 months old 5XFAD mice (**Fig. 4**). Immunoblotting showed no difference in levels of synaptic or axonal proteins in 5XFAD vs WT mice (**Fig. 5**).

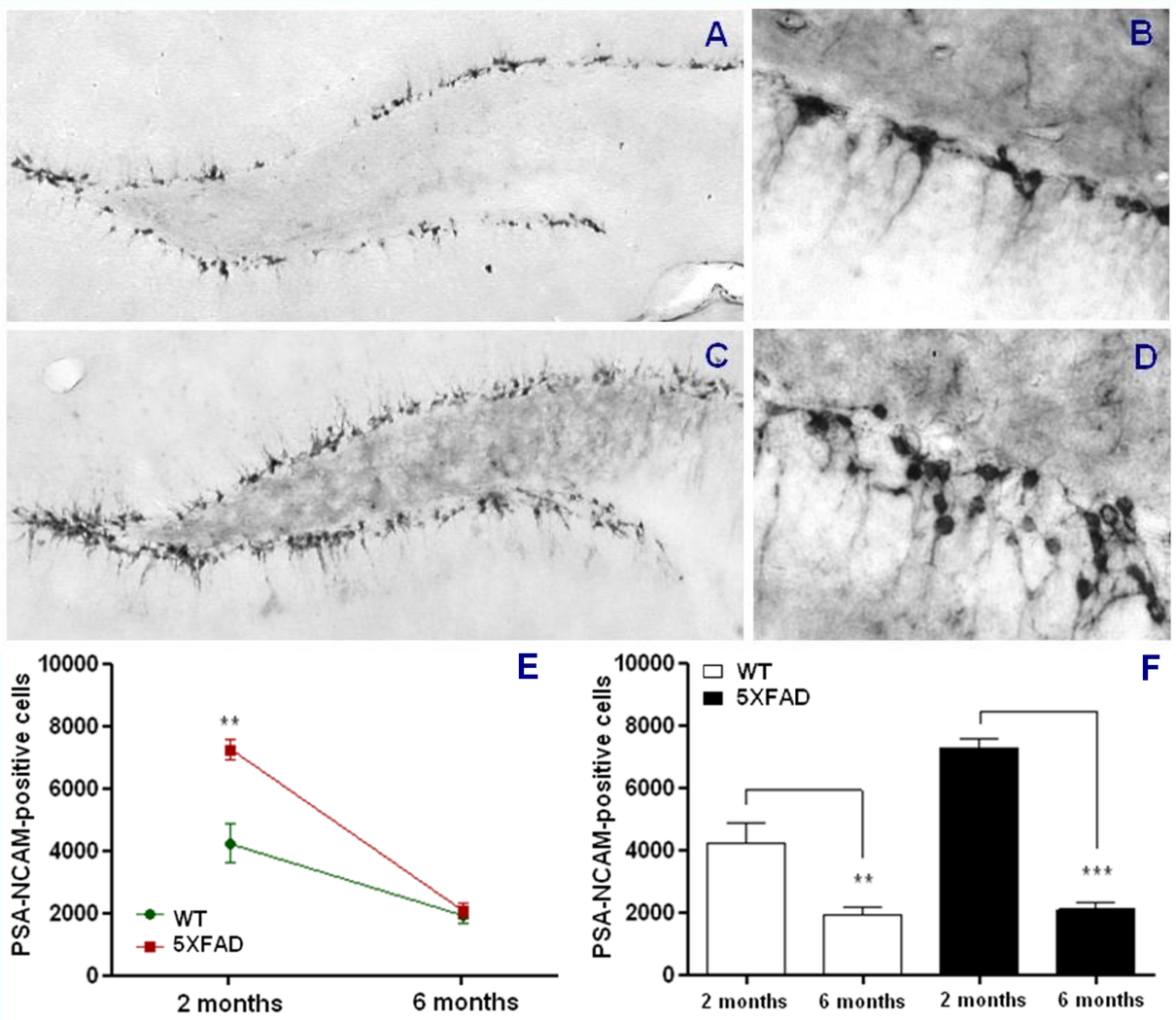

Figure 4. Light microscope images of PSA-NCAM positive cells in the hippocampal subgranular zone (SGZ) of 2 months old wild-type (**A, B**) and 5XFAD mice (**C, D**). Magn. 40 x (**A, C**) and 60 x (**B, D**). **E, F –** stereologically estimated total number of PSA-NCAM-positive cells in the hippocampal SGZ of 2 and 6 month old 5XFAD and wild-type mice. **p<0.01,***p<0.001, t-test. Immunoblotting showed similar hippocampal levels of NCAM and significantly higher levels of PSA-NCAM in 6 month old and a trend for increase in 2 months old 5XFAD mice (not shown).

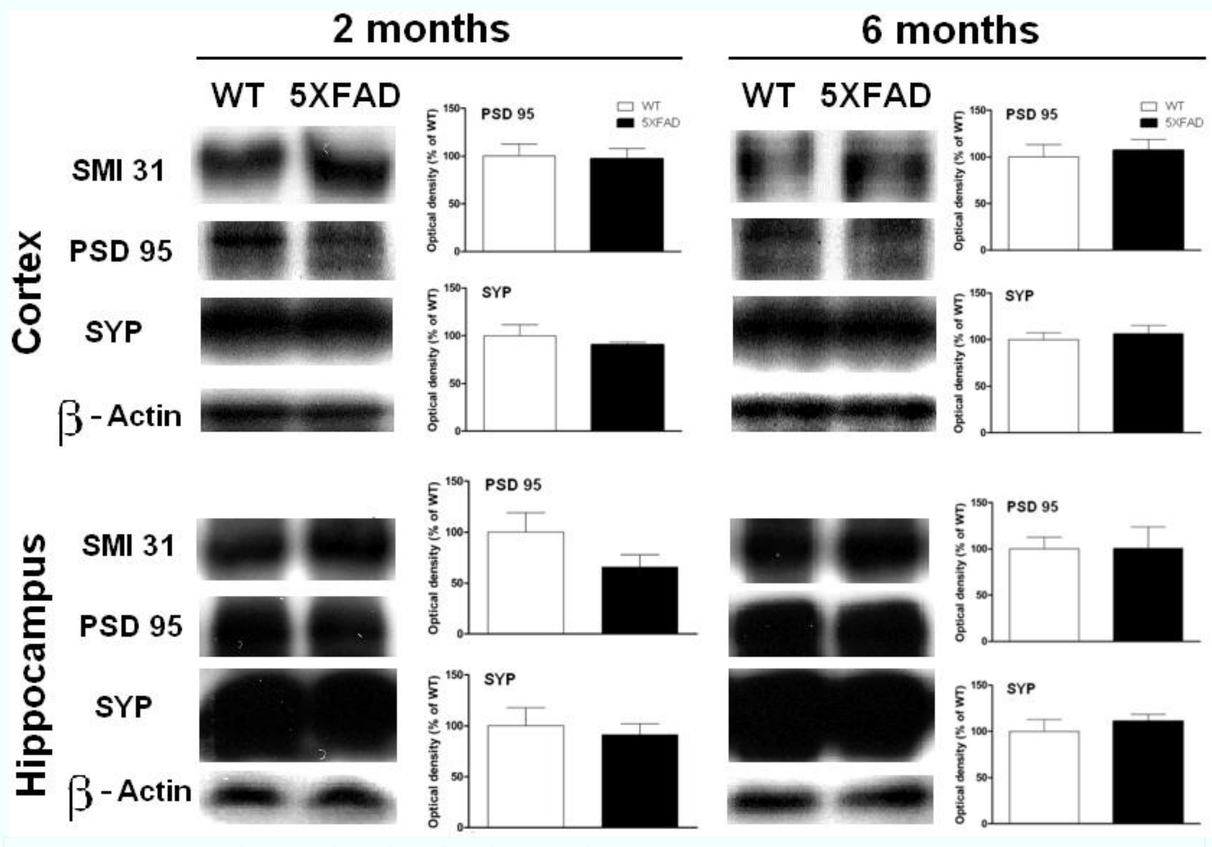

Figure 5. Immunoblots of phosphorylated neurofilament H (SMI31, 1:2500, Covance), postsynaptic density protein 95 (PSD95, 1:2000, Abcam) and synaptophysin (SYP, 1:10000, Santa Cruz) in frontal/prefrontal cortex and hippocampus of 5XFAD and WT mice at 2 and 6 months of age. These target proteins are known to be altered in AD([**2**]-[**6**]). Levels of proteins are normalized to β-actin (1:10000, Abcam) and expressed as a % of WT SEM. Ratio of phosphorylated to nonphosphorylated neurofilament was calculated additionally. Immunoblot and stats for nonphosphorylated neurofilament (SMI32) levels and ratio SMI31/SMI32 are not shown.

Conclusions

Despite progressive amyloidosis and microgliosis in the brain of 5XFAD mice, no progressive cognitive impairment or evidence of neuronal damage could be seen. Compensatory phenomena such as increased neurogenesis or excessive synthesis of neuronal proteins may exist. Rodent models do not necessarily recapitulate all brain/behavioural changes occurring in human AD, but nevertheless our findings question the validity and usefulness of this AD model for drug disvovery.

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