D-SERINE CONTRIBUTES TO β-AMYLOID-DEPENDENT PATHOPHYSIOLOGY IN ALZHEIMER'S DISEASE

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Key regulators of the structural and functional brain plasticity, the N-methyl-D-aspartate subtype of glutamate receptors (NMDARs) requires the binding of the co-agonist D-serine to be activated. In Alzheimer's disease (AD), soluble oligomers of the beta-amyloid peptide (Aßo) affect NMDARs possibly through mechanisms involving changes in D-serine levels since Aßo stimulate *in vitro* the production of the co-agonist. In this study, we asked whether D-serine contributes *in vivo* to morpho-functional NMDAR-related deregulations mediated by Aßo. Behavioral analysis combined to electrophysiological recordings at CA1/CA3 hippocampal synapses have been thus conducted in the 5xFAD transgenic mice model of amyloïdogenesis displaying marked increase in Aßo rates and compared to 5xFAD animals in which the homozygous gene of the serine racemase (SR) that synthesizes D-serine, has been jointly invalidated.

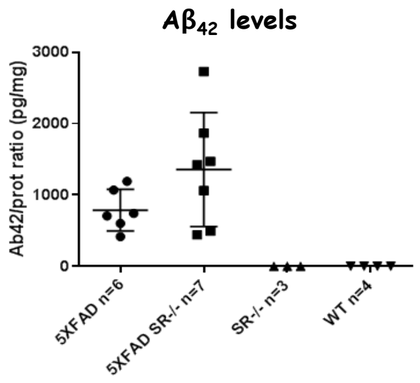
EXPERIMENTAL PROCEDURES

1) Behavioral analysis: 8-min spontaneous alternation test was performed in a Y maze apparatus to assess working memory performances in 10-12 months of aged mice. Successive entry of the three arms of the maze was considered as an alternation. The percentage of alternation was calculated as follows: number of alternations / (total number of arms visited – 2) x 100.

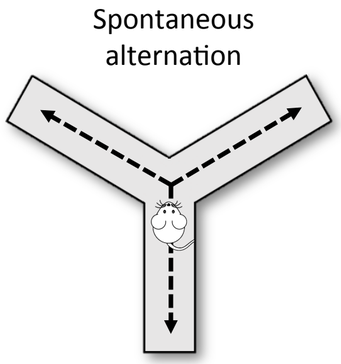
2) Electrophysiology: Hippocampal slices (400 µm thickness) were cut from two groups of WT, 5xFAD/SR$^{+/+}$ and 5xFAD/SR$^{-/-}$ mice aged 3-4 or 10-12 months. Field excitatory postsynaptic potentials (fEPSPs) and presynaptic fiber volley (PFV) were extracellularly recorded in CA1 stratum radiatum after electrical stimulation of Schaffer collaterals. Input/output curves of the fEPSP/PFV ratio of isolated NMDAr-mediated fEPSPs were constructed in a low magnesium medium supplemented with the non-NMDAr antagonist NBQX (10µM) before and 15 min after addition of D-serine (100 µM). High frequency (HFS)-induced long-term potentiation (LTP) was studied in control medium after tetanic stimulation consisting in one train at 100 Hz delivered for 1 sec. Testing stimulation was then resumed for 60 min after HFS.

3) Semi-quantitative immunoblotting analysis: Hippocampal tissue was homogenized in protein lysis buffer. The membranes were probed with antibodiesaginst GluN1 (1:750), GluN2A (1:2500), GluN2B (1:800), GluA2 (1:500), serine racemase (1:400) or ß-actin (1:7000). Proteins bands of interest were analyzed by scanning densitometry and normalized to ß-actin density.

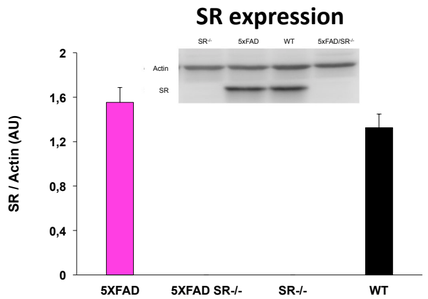
Hippocampal phenotype of the experimental groups | **Behavioral analysis** | **Functional plasticity (HFS-induced LTP)** | **NMDAR activation (isolated synaptic potentials)** | **Protein expression**

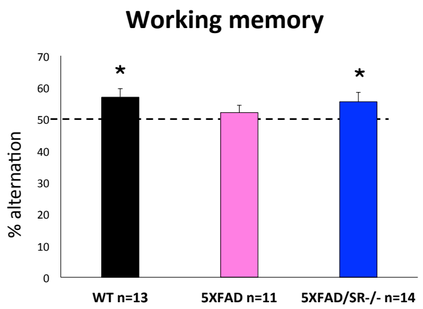


When in WT and SR$^{-/-}$ mice displayed only traces of Aßo, significant levels are found in both 5xFAD and 5xFAD/SR$^{-/-}$ animals

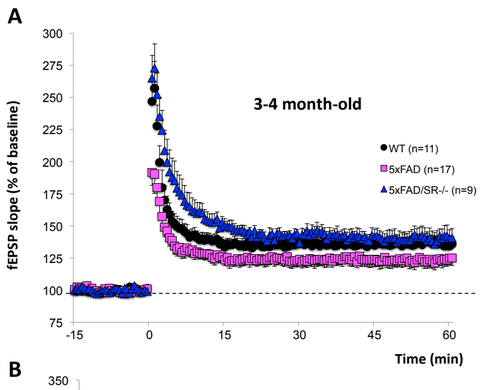


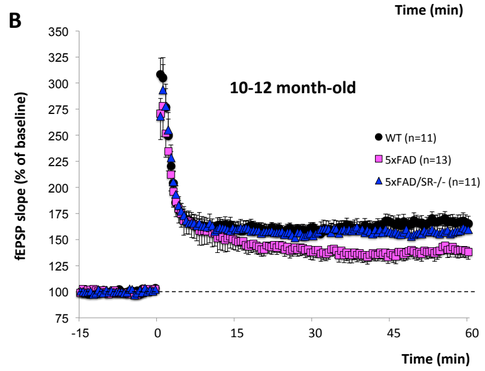
Y maze



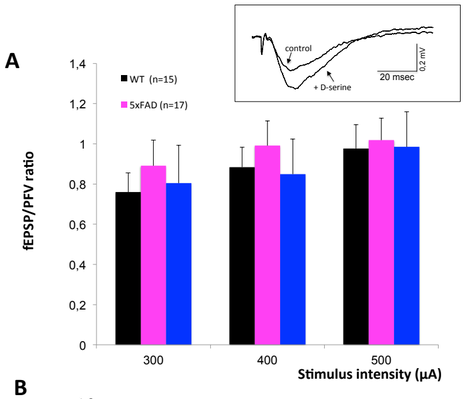


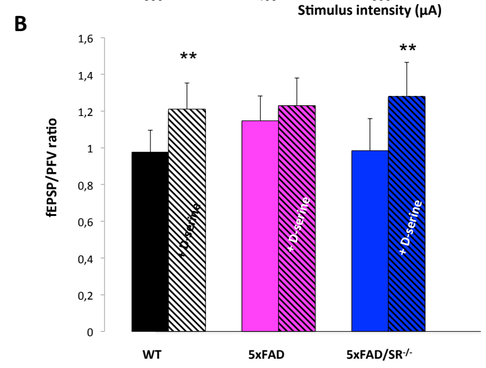
SR deletion reverses working memory deficits displayed by 5xFAD mice



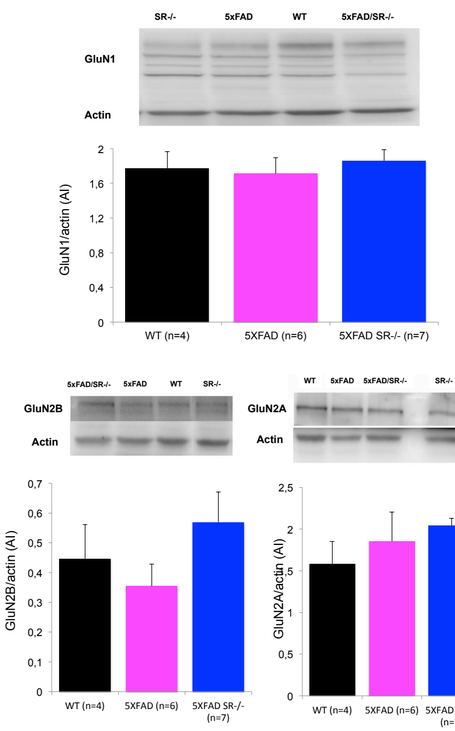


SR deletion reverses LTP deficits displayed by both young (A) and aged (B) 5xFAD mice





Low frequency-induced NMDAR activation is similar in basal conditions in all experimental groups (A) but is significantly increased by exogenous D-serine only in WT and 5xFAD/SR$^{-/-}$ mice (B)



Total protein expression for each NMDAR subunit is not significantly affected in 5xFAD mice although it is slightly decreased for GluN2B as compared to WT and 5xFAD/SR$^{-/-}$ mice

Our results therefore show that deletion of serine racemase prevents memory-related behavioral deficits observed in mice with prominent features of amyloidogenesis as well as impairment of NMDAR-dependent functional plasticity, suggesting a significant contribution of D-serine in NMDAR-dependent β-amyloid-related pathophysiology of Alzheimer's disease.