

Additional file 1. **A β ₁₋₄₂ oligomers are found in the hippocampus 1 and 24 hours after a single *icv.* injection**

Western blot protocol

Since behavioral and electrophysiological experiments were carried out from 1 hour to 17 days post-*icv.* A β ₁₋₄₂ administration, Western blot was performed to determine the possible A β oligomers clearance.

Briefly, hippocampal tissue samples at different time points (1 h, 24 h and 17 days; n = 4-7 per group) were homogenized in ice-cold RIPA lysis buffer (50 mM Tris-HCl pH 7.4; 150 mM NaCl; 0.1% Tx100; 0.5% sodium deoxycholate; 0.1% SDS). Equal amounts of protein (30 μ g) were mixed with Laemmli buffer (Bio-rad, USA), then loaded on an SDS-PAGE gel and subjected to electrophoresis. Proteins were transferred to nitrocellulose membranes (Bio-rad, USA) using a transblot apparatus (Bio-Rad, USA). Membranes were blocked with 5% dried skimmed milk powder in Tween-PBS for 1 h to prevent non-specific binding and incubated with the primary antibody against A β ₁₋₄₂ (rabbit monoclonal, 1/1000; Abcam, UK) overnight at 4 °C. After washing, antirabbit HRP-conjugated secondary antibody was added at a dilution of 1/5000 and incubated for 1 h. Blots were washed again, incubated in enhanced chemiluminescence reagent (ECL Prime; Bio-rad, USA), and developed using the G:BOX Chemi XX6 gel documentation system (Syngene, India). Expected molecular weights are 4 kDa for monomers, $\approx$ 50 kDa for oligomers and higher molecular weights (100-200 kDa) for insoluble fibrils (55). For blot quantification, density of the $\approx$ 50 kDa band was determined using ImageJ software (ImageJ, USA).

A primary antibody against β -actin (mouse monoclonal, 1/5000, 42 kDa; Sigma-Aldrich, USA) and appropriate secondary antibody (antimouse HRP-conjugated, 1/5000) was used as a loading control. Values were expressed as the ratio of the protein of interest/ β -actin and in percentage of the control group (100%).

A β ₁₋₄₂ clearance after a single *icv.* injection

Our results revealed that 1 hour after treatment, both male and female treated with A β ₁₋₄₂ exhibited significantly higher levels of the peptide in the hippocampus compared to vehicle-treated mice (Figure S1; treatment effect: $F_{(1,19)} = 52.59$, $p < 0.0001$; sex effect: $F_{(1,19)} = 0.8533$, $p = 0.3672$), confirming successful deposition of the peptide

following a single *icv.* injection. The increased presence of $A\beta_{1-42}$ in the hippocampus was still evident at 24 hours post-injection (Figure S1; treatment effect: $F_{(1,19)} = 9.582$, $p < 0.006$; sex effect: $F_{(1,19)} = 0.1458$, $p = 0.7068$). However, by 17 days after *icv.* injection (corresponding to the last day of behavioral and electrophysiological analysis in our study), $A\beta_{1-42}$ levels were comparable between the treatment and control groups (Figure S1; treatment effect: $F_{(1,14)} = 0.08071$, $p < 0.7805$; sex effect: $F_{(1,14)} = 0.6543$, $p = 0.4321$). This indicates that by day 17 post-*icv.* administration, clearance of the peptide has occurred, although the detrimental effect on synaptic function persists, as demonstrated by the memory and electrophysiological deficits observed.

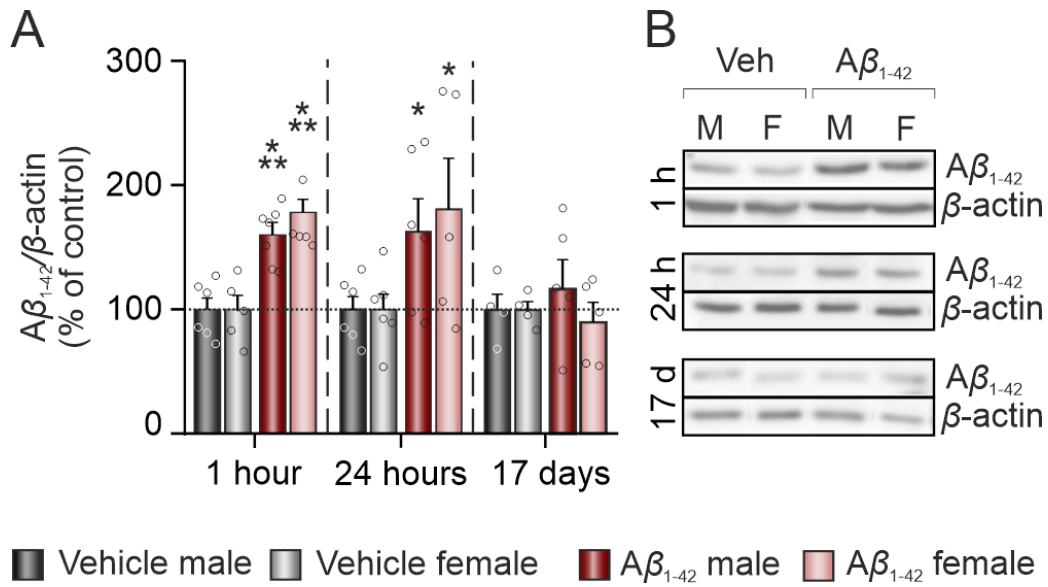


Figure S1. Hippocampal $A\beta_{1-42}$ expression at different time points after *icv.* treatment. (A) Relative expression of $A\beta_{1-42}$ in vehicle and $A\beta_{1-42}$ treated mice 1 hour, 24 hours and 17 days post-*icv.* administration. Data is expressed as the mean $\pm$ SEM and as percentage (%) of the controls. **(B)** Representative western blots of $A\beta_{1-42}$ and β -actin, as loading control, in each group. N vehicles: males = 4-6 and females = 4-6; N $A\beta_{1-42}$: males = 5-7 and females = 5. $A\beta$, Amyloid- β ; d, day; F, female; h, hour; *icv.*, intracerebroventricular; M, male; veh, vehicle. * $p < 0.05$, *** $p < 0.001$ vs. vehicle of the corresponding sex and time point.