Supplementary informations

Title

Low hippocampal PI(4,5)P2 contributes to reduced cognition in old mice due to loss of MARCKS

Authors

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Supplementary Figure 1: Age-associated changes in the levels of PI(4,5)P2, PI(3,4,5)P3, Akt and in extrasynaptic material.

(a) Levels of PI(4,5)P2 and PI(3,4,5)P3 in the extrasynaptic material from 10-MO and 20-MO mice compared to 4-MO mice (PIP2: 4MO=0.98 ±0.05, 10MO=0.96 ±0.07 20M=1.23 ± 0.32 STD, p4mo-20mo= 0.18 p10mo-20mo= 0.24 using a two-tailed t-test n=4 animals each age; PIP3: 4MO=0.89 ±0.22, 10MO=0.7 ±0.27 20M=0.89 ± 0.34 STD, n= 4 animals each age. Values are expressed as a ratio young versus old . (b) Levels pAkt in 10-MO and 20-MO mice compared to 4-MO mice (4MO=1, 10M=0.97 ± 0.4 STD 20MO=0.98 ± 0.4 STD; n=3-4 animals each age). The values are expressed as a ratio (4MO=1).
Supplementary Figure 2: Age-associated changes in phosphoinositides homeostasis

(a) Levels of IL-1β in the hippocampi of aged mice (4MO=0.23 pg/ml ± 0.06 STD, 20MO=0.59 pg/ml ± 0.07 STD, p=0.03, normalised to β-actin of protein n= 4-5 animals each age). (b) Levels of the active phosphatase PTEN in the hippocampi of mature and old mice (4MO=1.00 ± 0.04 STD, 20MO=1.10 ± 0.04 STD, p=0.276 n=3 animals each age). The values are expressed as a ratio (4MO=1). (c) PI(3,4,5)P3 reduction and PI(4,5)P2 increase after incubation with 100 μM LY294002 (PIP3: Ctrl=1.08 ± 0.33 STD, LY294002=0.11 ±
0.07 STD p=0.008 n=3 animals each condition; PIP2: Ctrl=1.08 ± 0.39 STD, LY294002=1.88 ± 0.24 STD p=0.04 n=3 animals each condition). (d) Synaptic/non-synaptic PI(4,5)P2 levels in hippocampal neurons maintained in vitro for different lengths of time (15DIV=0.17± 0.07 STD, 24DIV=0.09 ± 0.03 STD, p=0.01 n=10 cells for 3 independent experiments for each age). Boxed areas in the upper photographs are shown enlarged in the bottom of each panel.
Supplementary Figure 3: AAV-mediated MARCKS over-expression in the hippocampi.

(a) Overview of the diffusion of injected AAV in the hippocampus. (b) AAV-GFP MARCKS was largely restricted to neurons (NeuN + cells), (c) sparing glial cells (GFAP + cells). (d) Ratio GFP-positive cells versus the total NeuN-positive cells (0.27± 0.06 STD).
Supplementary Figure 4: Age-associated changes in the ratio of cholesterol/sphingomyelin in hippocampal synapses.

(a) Change in the molar ratio of cholesterol versus sphingomyelin in the synaptosomes from old mice (4M = 8.9 ± 0.54 STD, 20M = 8.02 ± 0.28 STD, p = 0.042 using a two-tailed t-test n=3 animals each age).
Supplementary Figure 5: Full-length pictures of the blots presented in the main figures.

ORP9: Oxysterol-binding protein-related 9; TrkB-tr: TrkB truncated form; N.S: not specific band.