

High levels of 27-hydroxycholesterol results in synaptic plasticity alterations in the hippocampus

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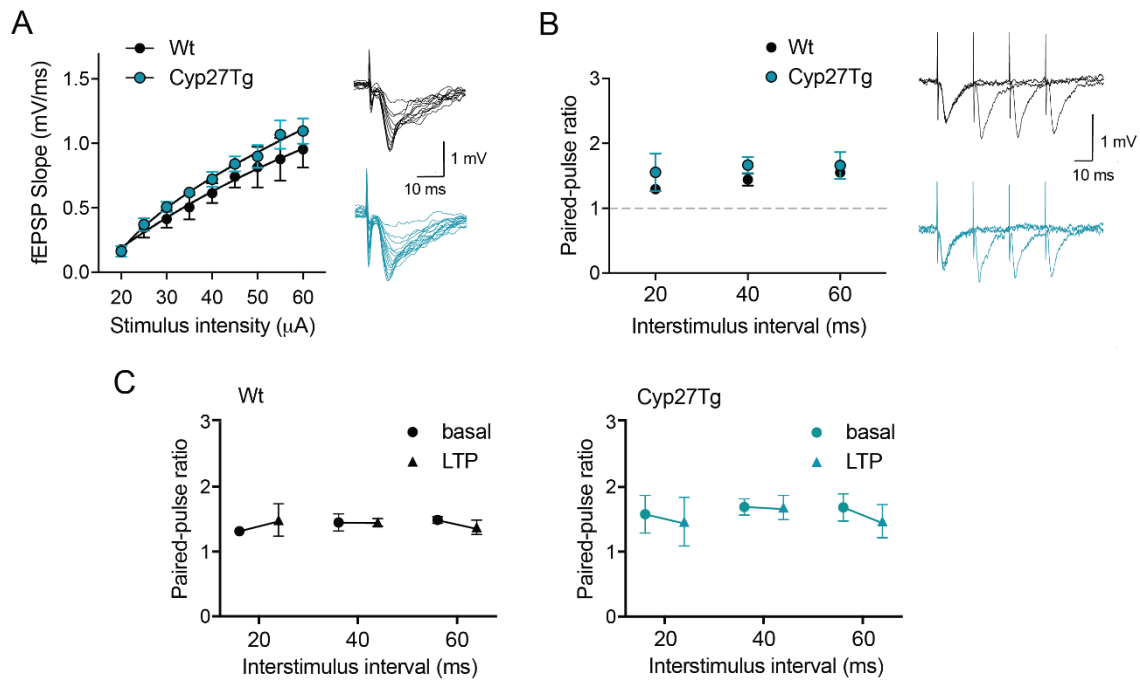
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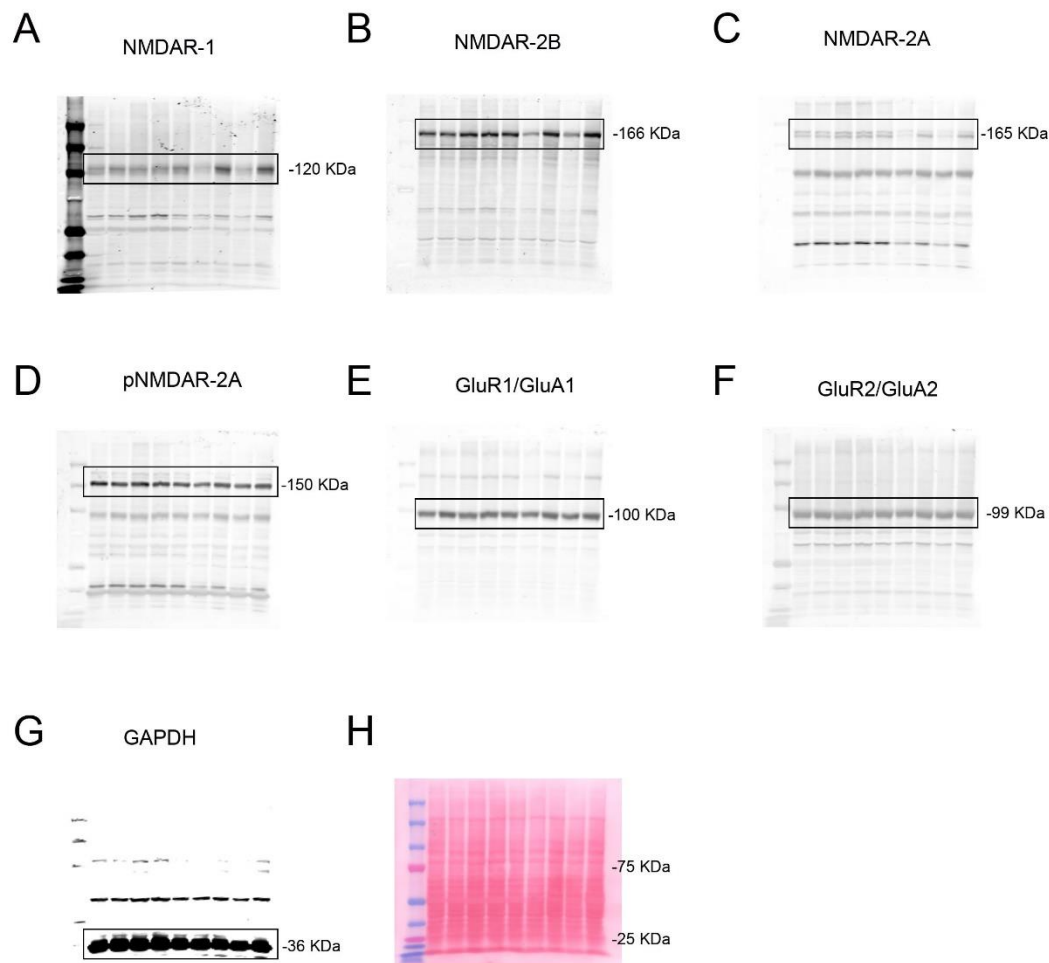
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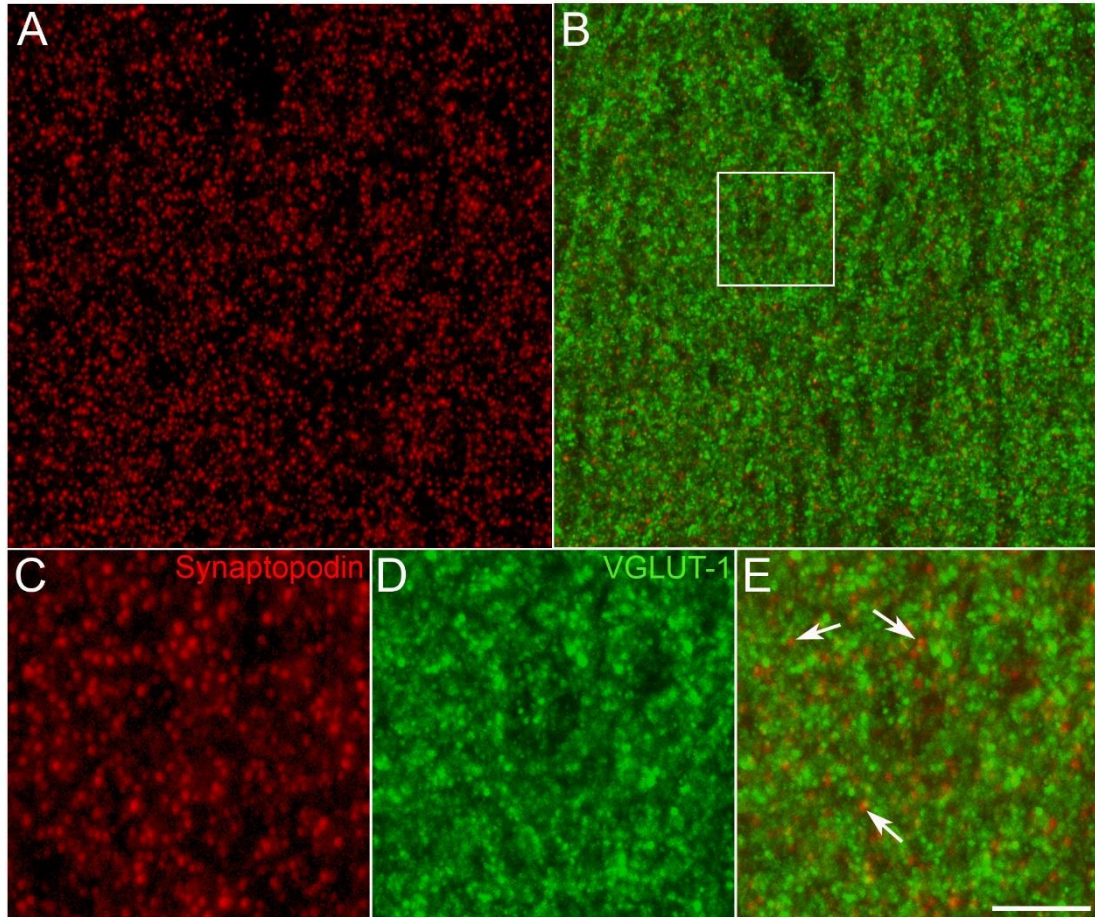
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Supplementary Figure 1. Analysis of the input-output included in Figure 1. (A) Input-output relationships showing the fEPSP slope as a function of stimulus intensity. Two-way repeated measures ANOVA showed no significant difference between Wt and Cyp27Tg mice ($F(1,8)=0.87$, $P=0.38$). (B) Paired pulse facilitation expressed in terms of the ratio (fEPSP2/fEPSP1) at 20, 40 or 60 ms interstimulus intervals showed no significant differences between genotypes (Two-way RM ANOVA $F(1,4) = 0.0004$, $P=0.98$). (C) LTP did not further modified significantly the paired pulse ratio at the tested interstimulus intervals in Wt (Two-way RM ANOVA $F(1,2) = 0.06$, $P=0.82$) or Cyp27Tg mice (Two-way RM ANOVA $F(1,2) = 4.11$, $P=0.18$).



Supplementary Figure 2. Full-length images for the western blots included in Figure 2. Western blot analysis of the glutamate receptor N-methyl-D-aspartate receptor subunits: (A) NMDAR-1, (B) NMDAR-2B, (C/D) NMDAR-2A/pNMDAR-2A; and receptor subunits of the 2 α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors: (E) GluR1/GluA1 and (F) GluR2/GluA2. (G) GAPDH was used as control and (H) Ponceau S staining as a quality control test. Black boxes indicate the cropped section as displayed in the main body figure.



Supplementary Figure 3. Double immunofluorescence images using antibodies for synaptopodin and VGLUT-1 in the *stratum radiatum* of a WT mouse. (A, C) Confocal images (63x objective; NA, 1.4; image resolution 1024×1024 pixels) showing synaptopodin immunoreactive puncta to be quantified as shown in Figure 5. (B, D) Staining for the presynaptic marker VGLUT-1. (E) Merge of C and D. Note the lack of colocalization of immunoreactivities for synaptopodin and VGLUT-1. Arrows indicate some synaptopodin puncta adjacent to VGLUT-1 puncta. Scale bar shown in E indicates 15 μm in A- B and 5 μm in C-E.