Supplemental Material for

Title: GluA2 (GluR2) Regulates Metabotropic Glutamate Receptor-Dependent Long-Term Depression through N-cadherin-Dependent and Cofilin-Mediated Actin Reorganization

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Supplemental Figure Legend

Supplemental Figure 1. Characterization of DHPG-induced LTD.

(A) Inhibition of DHPG-induced LTD by the group I mGluR antagonist MPEP (10 μM) perfused in ACSF throughout the experiment.

(B, C) Whole-cell recordings of AMPAR-mediated EPSCs in the presence of APV (100 μM) showing DHPG-induced LTD (B), but without changes in rectification indices before and after DHPG application (C).

(D) Whole-cell recordings showing DHPG-induced LTD of NMDAR-mediated EPSCs in the presence of the AMPAR antagonist NBQX (10 μM). This experiment was done to show that DHPG induced LTD (at least for NMDAR-mediated component of synaptic response) does not require activation of AMPARs. To achieve this, we blocked AMPAR-mediated responses completely (with NBQX) and recorded NMDAR-mediated EPSCs and then applied DHPG to induce LTD. Since LTD of NMDAR-mediated responses can still be produced, the data suggest that DHPG-LTD does not require activation of AMPARs.

(E) Schematic map of viral vectors used for virus production and in vivo injection. Except for control EGFP vector in which only the EGFP cassette was present, all other vectors contained both EGFP and various HA- or Myc-tagged GluA2 cDNA in two independent expression cassettes.
(F) Whole-cell recordings showing lack of effect of expressing A2+EGFP on DHPG-induced LTD in WT neurons.

(G, H) I/V curves of AMPAR-mediated EPSCs (G) and averaged rectification indices (H) of WT CA1 neurons infected with (A2+EGFP) or without (WT) GluA2 plus EGFP viruses showing lack of effect of expressing A2+EGFP in WT neurons.

Supplemental Figure 2. Effect of INP peptide on basal synaptic responses and DHPG-induced changes in tyrosine-phosphorylated β-catenin.

(A) Field EPSP recordings showing blockade of DHPG-induced LTD by pre-treating the slices with the N-cadherin inhibitory peptide INP (50 μM, 30 min), but not by the control DMSO (50 μM, 30 min).

(B) Field EPSP recordings showing lack of effect for INP peptide (50 μM, 30 min) on basal synaptic transmission.

(C) Field EPSP recordings showing lack of effect for INP peptide (50 μM, 30 min) on DHPG-induced LTD when applied after DHPG application.

(D) Western blot analysis of protein lysate prepared from hippocampal slices immunoprecipitated with anti-phosphorylated tyrosine antibody and probed with anti-β-catenin showing a dramatic increase in the basal level of tyrosine phosphorylated β-catenin in GluA2 KO mice.
(E) Western blot analysis of protein lysate prepared from hippocampal slices treated with (5 min) or without (0 min) DHPG, immunoprecipitated with anti-phosphorylated tyrosine antibody and probed with anti-β-catenin showing a significant increase in the amount of tyrosine phosphorylated β-catenin by the treatment in WT, but not in GluA2 KO samples.

**Supplemental Figure 3.** Lack of effect of phalloidin/latrunculin on hippocampal CA1 NMDAR-dependent LTD.

(A) Whole-cell recordings of AMPAR-mediated EPSCs showing lack of effect on NMDAR-dependent LTD for the actin inhibitor phalloidin (100 µM) and latrunculin A (200 µM). LTD was induced by 5 Hz low frequency stimulation (5Hz, 3 min) delivered at -30 mV holding potential.

(B) Whole-cell recordings of AMPAR-mediated EPSCs showing lack of effect on basal synaptic responses for phalloidin (100 µM) and latrunculin A (200 µM).

**Supplemental Figure 4.** ERK activation by DHPG in WT and GluA2 KO mice.

(A) Blockade of DHPG-induced LTD by the ERK1/2 MAPK signaling inhibitor PD98059 (50 µM), but not by the p38 kinase MAPK inhibitor SB203580 (5 µM).

(B) Western blot analysis of hippocampal protein lysate and summary graph showing a significant increase in phosphorylated (active) ERK1/2 in DHPG-treated slices in both WT and GluA2 KO mice. The total protein level of ERK1/2 is not altered by the treatment.
(C) Western blot analysis and summary graph of total and phosphorylated coflin using protein lysates prepared from hippocampal slices treated with DHPG in the presence of actinomycin D (25 µM) or cycloheximide (60 µM), showing lack of effect of blocking transcription or translation on DHPG-induced coflin activation.

(D) Whole-cell recordings showing the blockade of DHPG-induce LTD by protein synthesis inhibitor cycloheximide (60 µM) in GluA2/LIMK-1 double KO mice, indicating that protein synthesis-dependent mechanisms are still required in these mice.

**Supplemental Figure 5.** Mechanisms underlying DHPG-induced LTD shared by cultured hippocampal neurons and acute slices.

(A) Whole-cell recordings from cultured hippocampal neurons showing stable mEPSC amplitudes before and after DHPG application (solid line) in both WT and GluA2 KO neurons.

(B) Whole-cell recordings of mEPSCs of CA1 neurons in hippocampal slices showing significant and predominant reduction in the frequency, with minimal changes in the amplitude after DHPG treatment.

(C) Blockade of DHPG-induced LTD in cultured WT neurons by the protein synthesis inhibitor cycloheximide (60 µM), but not by the transcription inhibitor actinomycin D (25 µM).

(D) Blockade of DHPG-induced LTD in cultured WT neurons by ERK1/2 inhibitor PD98059 (50 µM), but not by the p38 kinase inhibitor SB203580 (5 µM).
(E) Blockade of DHPG-induced LTD in WT cultured neurons infused with the Rac1 inhibitor NSC23766 (250 µM), but not with the RhoA inhibitor C3 toxin (10 µg/ml).

(F) Blockade of DHPG-induced LTD in cultured WT neurons by postsynaptic inclusion of the actin stabilizer phalloidin (100 µM) or the actin polymerization inhibitor latrunculin A (200 µM).

(G) Blockade of DHPG-induced LTP in cultured WT neurons by postsynaptic infusion of the pS3 peptide (200 µM), but not by the S3 peptide (200 µM).

(H) Blockade of DHPG-induced LTD in cultured WT neurons by postsynaptic inclusion of anti-cofilin antibodies (10 µg/ml), but not by control IgG (10 µg/ml).

**Supplemental Figure 6.** A hypothetic model for GluA2/N-cadherin-dependent, cofilin-mediated actin reorganization in mGluR-LTD.

Under basal conditions, GluA2 forms a relatively stable complex with N-cadherin/catenin linked to the low-turnover actin cytoskeleton to maintain stable spine morphology and synaptic transmission. Upon mGluR activation, both GluA2 and catenins are released from the complex and from the actin cytoskeleton, likely through tyrosine phosphorylation/dephosphorylation of catenins. The released catenins cause activation of Rac1 and inhibition of RhoA (or rebalancing their activity), which collectively inhibit LIMK1 (or stimulate cofilin phosphatases) to activate cofilin. The activated cofilin promotes actin reorganization, which leads to morphological changes and/or elimination of spines/synapses, resulting in LTD.

Pre-disruption of GluA2/N-cadherin complex (e.g. by INP peptide or lack of GluA2)
leads to abnormal activation of cofilin, which occludes subsequent mGluR-dependent cofillin activation and LTD. Thus, the mGluR-LTD deficits in GluA2KO or INP treated slices are rescued by increasing cofilin activity. PTK/P: protein tyrosine kinase and phosphatase, p120/α/β: p120-, α-, β-catenins.
Supplementary Figure 1

A

\[ \begin{array}{c}
\text{Time (min)} \quad -20 \quad 0 \quad 20 \quad 40 \quad 60 \\
\% \text{EPSP slope} \quad 40 \quad 60 \quad 80 \quad 100 \quad 120 \\
\text{Ctrl (n=6)} \quad \text{MPEP (n=4)}
\end{array} \]

B

\[ \begin{array}{c}
\text{Time (min)} \quad -10 \quad 0 \quad 10 \quad 20 \quad 30 \quad 40 \\
\% \text{EPSC} \quad 40 \quad 60 \quad 80 \quad 100 \quad 120 \\
\text{Ctrl} \quad \text{MPEP}
\end{array} \]

C

\[ \begin{array}{c}
\text{Rectification Index} \quad 0.0 \quad 0.2 \quad 0.4 \quad 0.6 \quad 0.8 \quad 1.0 \\
\text{Time post-DHPG (min)} \quad 0 \quad 40 \\
\end{array} \]

D

\[ \begin{array}{c}
\text{Time (min)} \quad -10 \quad 0 \quad 10 \quad 20 \quad 30 \quad 40 \\
\% \text{EPSC} \quad 40 \quad 60 \quad 80 \quad 100 \quad 120 \\
\text{WT (n=5)} \quad \text{WT} \quad \text{WT} \quad \text{WT} \quad \text{WT}
\end{array} \]

E

\[ \text{pC4HSU} \quad \sim 30-35 \text{ kb} \]

pCMV
\[ \text{SV40 polyA} \]
\[ \text{EGFP} \]
\[ \text{HA- or Myc-tagged cDNA} \]
1. A2
2. A2-Δ92
3. A1-87-A2
4. A2-92-A1

F

\[ \begin{array}{c}
\text{Time (min)} \quad -10 \quad 0 \quad 10 \quad 20 \quad 30 \quad 40 \\
\% \text{EPSC} \quad 40 \quad 60 \quad 80 \quad 100 \quad 120 \\
\text{WT (n=6)} \quad \text{WT} \quad \text{WT} \quad \text{WT} \quad \text{WT}
\end{array} \]

G

\[ \begin{array}{c}
\text{Time (min)} \quad -80 \quad -60 \quad -40 \quad -20 \quad 0 \quad 20 \quad 40 \quad 60 \\
% \text{EPSC amplitude} \quad -1.5 \quad -1.0 \quad -0.5 \quad 0.0 \quad 0.5 \quad 1.0
\end{array} \]

H

\[ \begin{array}{c}
\text{Rectification index} \quad 0.0 \quad 0.2 \quad 0.4 \quad 0.6 \\
\text{WT} \quad \text{WT} \quad \text{WT} \quad \text{WT}
\end{array} \]
Supplementary Figure 2

A

B

C

D

E

WT
DHPG
KO
DHPG
0            5
0            5

Normalized p-β-catenin ratio

WT (n=5)          KO (n=5)

Normalized p-β-catenin ratio

WT (n=5)          KO (n=5)
Supplementary Figure 3

A

B
Supplementary Figure 5