Supporting Information

Flores et al. 10.1073/pnas.1411170112

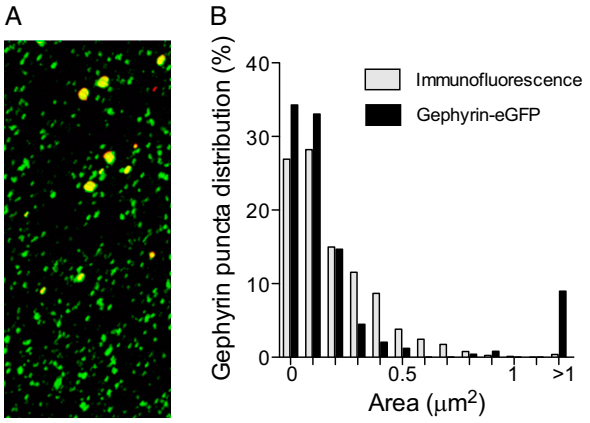

Fig. S1. Size analysis of gephyrin clusters. (*A*) Colocalization (yellow dots) of gephyrin immunofluorescence (green dots) detected in the CA1 stratum radiatum of a hippocampal slice culture and of gephyrin-eGFP clusters (in red for display purposes) expressed in a single transfected pyramidal neuron. Green dots correspond to gephyrin clusters revealed in nontransfected neurons. (*B*) Correlated histograms illustrating the size distribution of gephyrin clusters revealed by immunofluorescence in nontransfected neurons and the size distribution of gephyrin-eGFP clusters observed in transfected neurons. Note that the mean cluster size of the two distributions is not significantly different (unpaired *t* test).

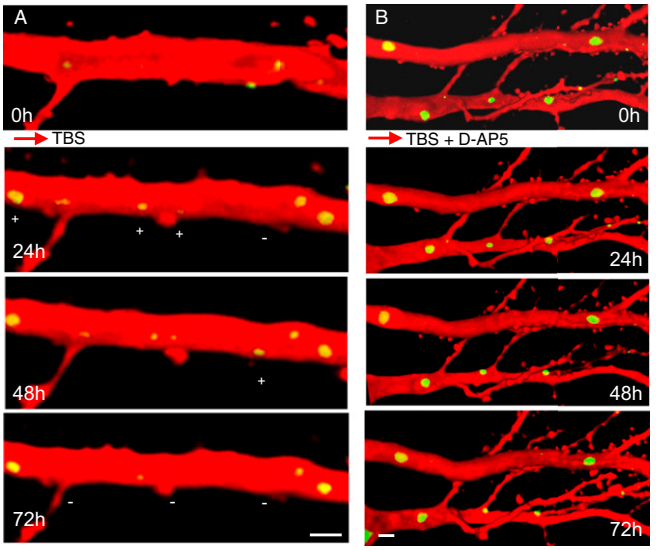

Fig. S2. Blockade of activity-induced gephyrin cluster dynamics by the NMDA receptor antagonist D-AP5. (*A*) Illustration of the changes in gephyrin cluster dynamics induced by TBS over 72 h. (*B*) Absence of changes in cluster dynamics over 72 h when TBS was applied in the presence of D-AP5 (50 μM). (Scale bar: 2 μm.)

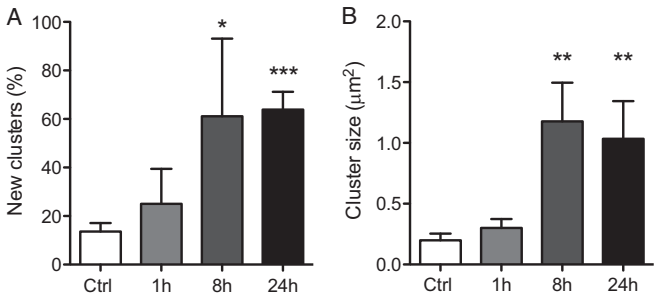

Fig. S3. Time course of changes in gephyrin cluster dynamics induced by gabazine. (*A*) Proportion of new gephyrin clusters detected 1, 8, and 24 h after gabazine treatment and compared with the fraction of new clusters per 24 h occurring without gabazine treatment ($n = 3$; unpaired t test). (*B*) Changes in gephyrin cluster size observed under the same conditions (unpaired t test). $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

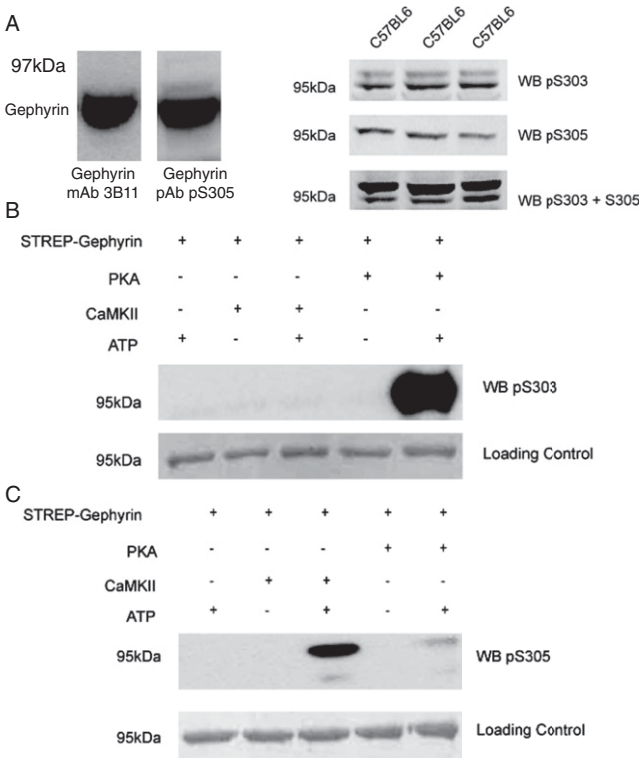

Fig. S4. Phosphorylation of gephyrin S303 and S305 sites by PKA and CaMKII. (*A*) Western blot detection of gephyrin from rat organotypic hippocampal cultures (*Left*) using a mouse monoclonal antibody against gephyrin (3B11, SYSY) and rabbit phospho-specific antibody against S305 on gephyrin (Genscript). The blot at *Right* illustrates the specificity of the pS303 and pS305 antibodies in recognizing different forms of gephyrin from a brain extract. (*B*) In vitro kinase assay demonstrating the phosphorylation of gephyrin S303 site by PKA but not by CaMKII. (*C*) In vitro kinase assay demonstrating the phosphorylation of gephyrin S305 site by CaMKII, but not by PKA. Blots were stripped and probed with the mouse monoclonal antibody against gephyrin to show equal levels of gephyrin.

Table S1. Quantitative analyses of gephyrin cluster dynamics and size observed within the next 24 h after application of the indicated treatments

Condition	New puncta (24 h)	*n* cells//puncta	Stat	Lost puncta (24 h)	Stat	Puncta size (24 h)	Stat
Control	15.0 ± 3.1	15//134	—	18.0 ± 3.8	—	0.29 ± 0.03	—
CCh	106.7 ± 21.4	4//24	$P < 0.001$	25.2 ± 6.6	ns	0.67 ± 0.11	$P < 0.01$
TBS	63.0 ± 12.0	7//75	$P < 0.001$	22.8 ± 1.6	ns	0.58 ± 0.07	$P < 0.001$
TBS+D-AP5	15.0 ± 4.9	6//62	ns	17.1 ± 4.9	ns	0.31 ± 0.05	ns
TBS+KN93	23.5 ± 5.3	7//47	ns	24.8 ± 5.9	ns	0.27 ± 0.07	ns
GBZ	89.4 ± 14.5	7//36	$P < 0.001$	21.6 ± 8.2	ns	0.74 ± 0.18	$P < 0.01$
ChR2-red light	16.9 ± 4.4	4//36	—	25.1 ± 4.6	ns	0.27 ± 0.07	—
ChR2-blue light	57.3 ± 20.1	6//86	$P < 0.05$	27.3 ± 7.9	ns	0.50 ± 0.06	$P < 0.01$
ChR2-blue light-NBQX-AP5	50.0 ± 10.3	3//82	$P < 0.05$	20.3 ± 7.0	ns	0.42 ± 0.04	$P < 0.05$
ChR2-blue light-TTX	65.1 ± 7.6	3//42	$P < 0.01$	36.1 ± 14.8	ns	0.50 ± 0.07	$P < 0.05$
SSA	12.4 ± 3.6	8//93	ns	36.5 ± 4.9	ns	0.24 ± 0.03	ns
SSA+TBS	8.9 ± 2.4	8//93	ns	22.4 ± 4.5	ns	0.30 ± 0.06	ns
S305A	24.9 ± 4.6	5//90	ns	39.8 ± 8.2	ns	0.31 ± 0.05	ns
S305A+TBS	11.9 ± 5.1	5//90	ns	36.5 ± 4.9	ns	0.31 ± 0.05	ns
SSD	42.8 ± 2.9	6//183	$P < 0.001$	38.4 ± 5.9	ns	0.35 ± 0.04	ns
SSD+TBS	53.6 ± 16.6	7//142	$P < 0.01$	26.1 ± 6.1	ns	0.48 ± 0.05	$P < 0.001$
S305D	37.0 ± 3.9	5//57	$P < 0.01$	23.1 ± 3.5	ns	0.30 ± 0.04	ns
S305D+TBS	61.7 ± 24.7	4//30	$P < 0.01$	34.5 ± 14.3	ns	0.32 ± 0.05	ns

New and lost clusters represent the fraction of newly formed and eliminated clusters detected over 24 h. Cluster size represents the mean surface in square micrometers of the preexisting clusters as observed 24 h after treatment. *n* indicates the number of cells (one per slice culture) and number of clusters analyzed. Statistics were carried out by using an unpaired *t* test.