Supplementary Information

Binding of the Complexin N terminus to the SNARE complex potentiates synaptic vesicle fusogenicity

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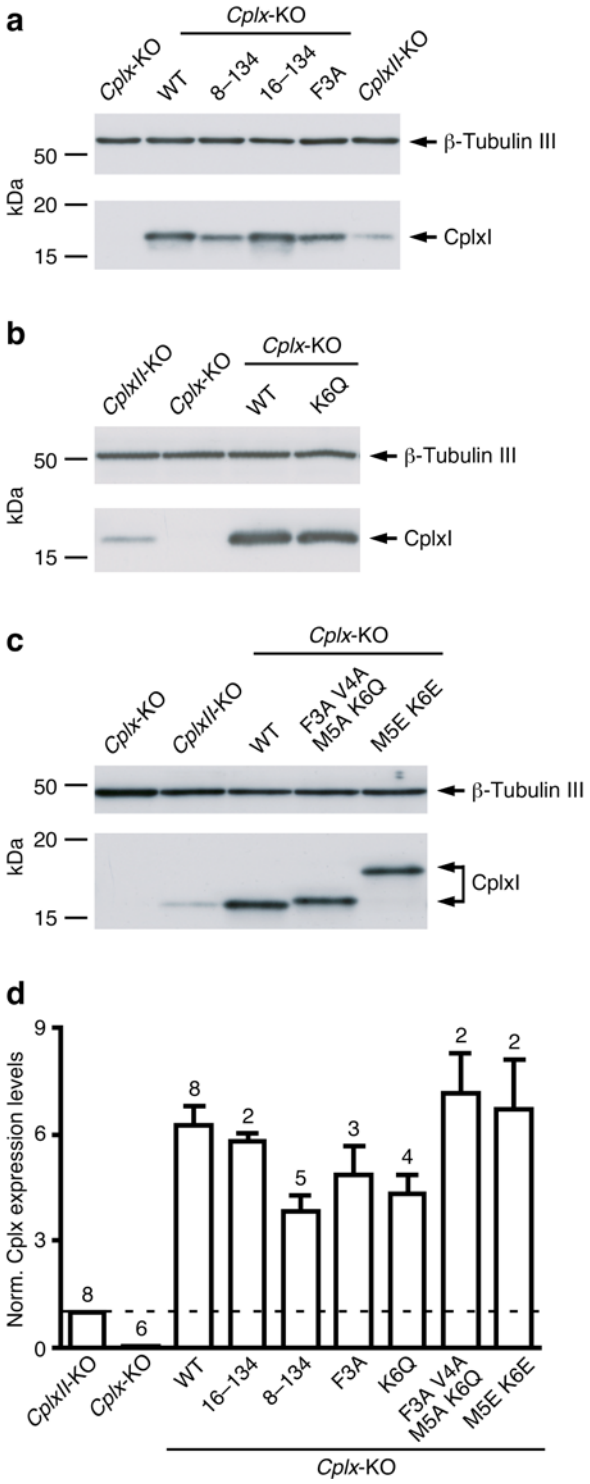
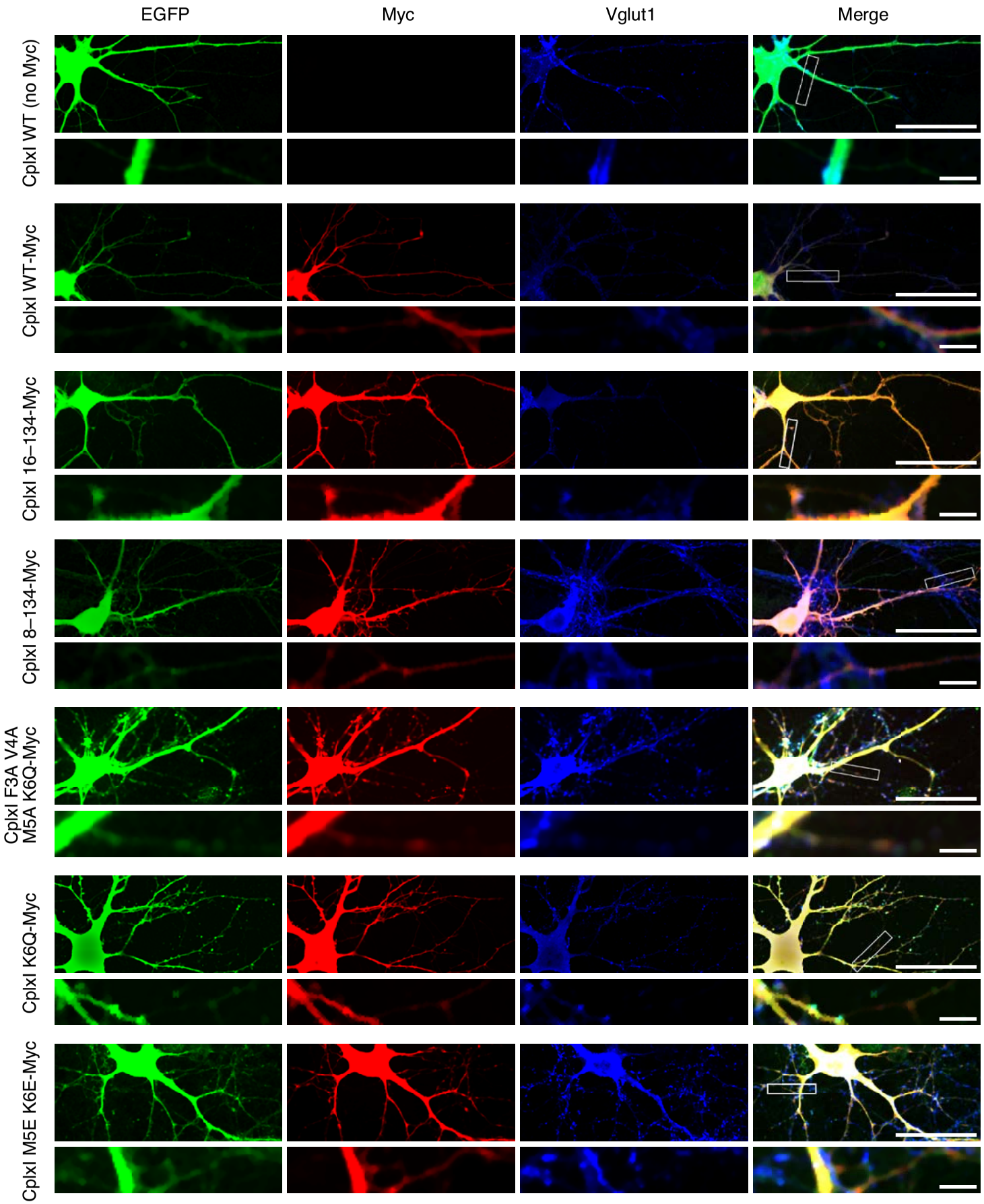
Supplementary Figure 1
Xue et al., 2010



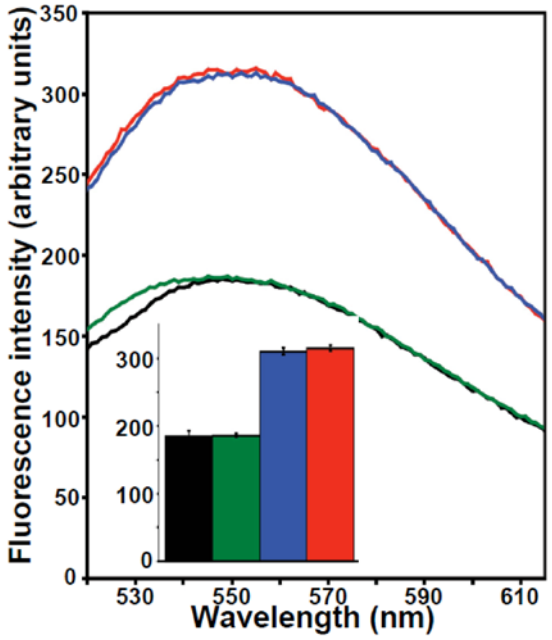
Supplementary Figure 1 SFV expressions of CplxI variants in hippocampal neurons. Proteins were extracted from mass-cultured hippocampal neurons 11–12 hours after virus infection and analyzed by Western blotting. The levels of viral expression of CplxI WT and mutants in *CplxI*/*II*-DKO or *CplxI*/*II*/*III*-TKO neurons (referred together as *Cplx*-KO neurons) were compared to the endogenous CplxI levels in *CplxII*-KO neurons. (**a–c**) Representative Western blots of *CplxII*-KO neurons, *Cplx*-KO neurons, and *Cplx*-KO neurons expressing CplxI variants. Protein extracts were immunoblotted with rabbit anti-CplxI/II (against C-terminus, 1:5000, Synaptic Systems) and mouse anti-β-Tubulin III (3B11, 1:5000, Synaptic Systems). (**d**) Bar graphs show the normalized expression levels of CplxI variants. CplxI expression levels were first normalized by the loading control neuronal β-Tubulin III. The expression levels of all CplxI in *Cplx*-KO neurons were normalized to the endogenous CplxI level in *CplxII*-KO neurons (dashed line). Data are expressed as mean ± SEM. The number of blots analyzed are indicated on the bars.

Supplementary Figure 2
Xue et al., 2010



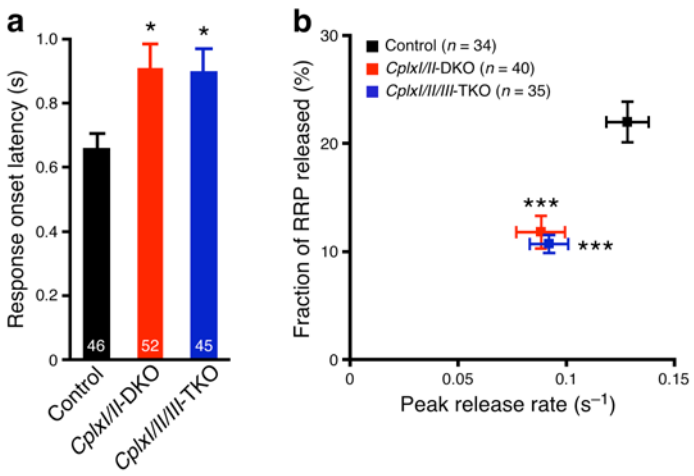
Supplementary Figure 2 Presynaptic localization of Myc-tagged CplxI variants in hippocampal neurons. Cultured hippocampal neurons were infected with SFV expressing CplxI-Myc variants and immunolabeled with chicken anti-GFP (1:500, Chemicon), mouse anti-Myc (9E3, 1:100, Santa Cruz), and rabbit anti-vesicular glutamate transporter-1 (Vglut1, 1:1000, Synaptic Systems) antibodies 10–11 hours after virus infection. Representative images show immunostainings of EGFP, Myc, and Vglut1. Merged images show the presence of CplxI-Myc variants in presynaptic termini labeled by Vglut1. Scale bars: 50 μm for low magnification (large images) and 5 μm for high magnification (small images).

Supplementary Figure 3
Xue et al., 2010



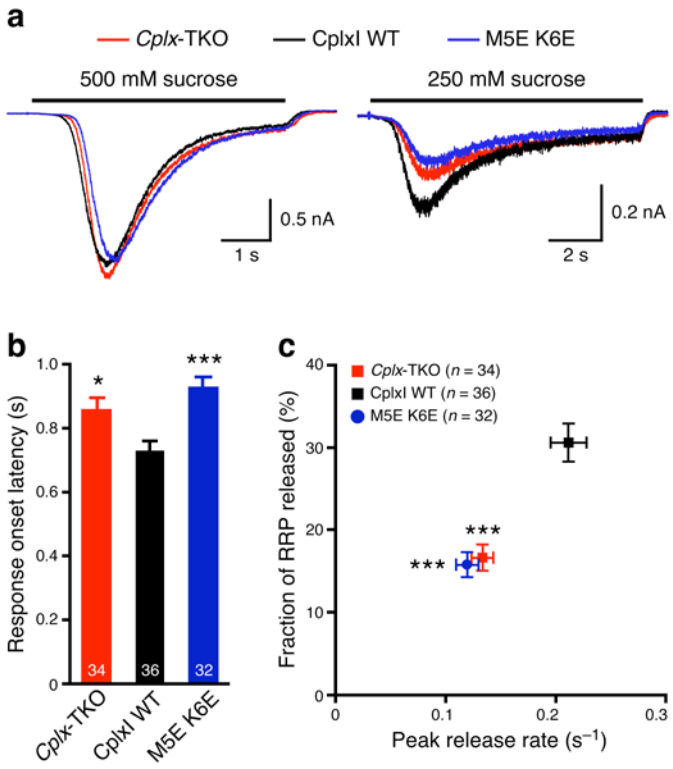
Supplementary Figure 3 Fluorescence experiments showing that the N terminus of CplxI binds to the soluble SNARE complex, but not to plain liposomes. To perform these experiments, we generated a CplxI mutant (CplxI A12C C105S) where Ala12 was mutated to cysteine, and the native cysteine (Cys105) was mutated to serine to ensure labeling of only residue 12. We covalently labeled the mutant protein at the cysteine residue with 7-nitrobenz-2-oxa-1,3-diazole (NBD). The diagram shows fluorescence spectra of NBD-labeled CplxI A12C C105S alone (black) and upon addition of plain liposomes (green), SNARE complex-containing proteoliposomes (blue), or the soluble SNARE complex (red). Representative traces from experiments repeated at least three times are shown. The bar graph in the inset shows mean fluorescence intensities at 550 nm ± standard deviation from three independent experiments performed under each condition.

Supplementary Figure 4
Xue et al., 2010



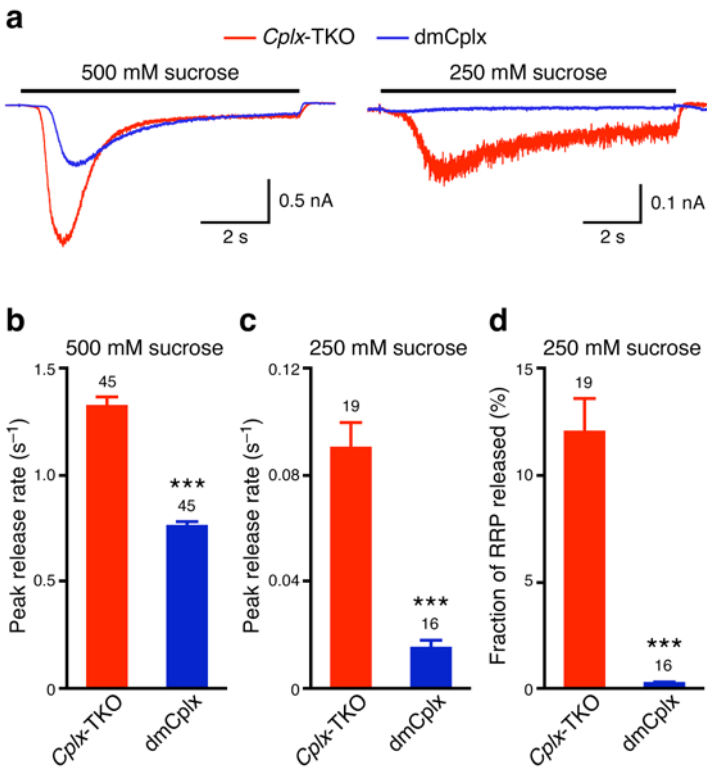
Supplementary Figure 4 Decreased synaptic vesicle fusogenicity in *Cplx*-deficient GABAergic neurons. Summary data of 250 mM sucrose solution-induced response onset latency (**a**), peak release rate and fraction of RRP released (**b**) from the striatal GABAergic neurons of control, *CplxI*/*II*-DKO, and *CplxI*/*II*/*III*-TKO mice. Data are expressed as mean ± SEM; *, $P < 0.05$; ***, $P < 0.001$ compared to control. Control neurons are *CplxII*$^{-/-}$. The numbers of neurons analyzed are shown in figures.

Supplementary Figure 5
Xue et al., 2010



Supplementary Figure 5 Complexins potentiate synaptic vesicle fusogenicity in mass-cultured hippocampal neurons. (**a**) Average traces of glutamatergic synaptic responses induced by sucrose solutions from *CplxI/II/III*-TKO (*Cplx*-TKO) neurons (500 mM, n = 34; 250 mM, n = 34), *Cplx*-TKO neurons rescued by CplxI WT (500 mM, n = 36; 250 mM, n = 36), and *Cplx*-TKO neurons rescued by CplxI M5E K6E (500 mM, n = 32; 250 mM, n = 32). (**b,c**) Summary data of 250 mM sucrose solution-induced response onset latency (**b**), peak release rate and fraction of RRP released (**c**) from *Cplx*-TKO neurons and *Cplx*-TKO neurons rescued by CplxI WT or M5E K6E mutant. Data are expressed as mean ± SEM; *, $P < 0.05$; ***, $P < 0.001$ compared to CplxI WT-rescued *Cplx*-TKO neurons. The numbers of neurons analyzed are indicated in the figures.

Supplementary Figure 6
Xue et al., 2010



Supplementary Figure 6 *Drosophila* Complexin decreases synaptic vesicle fusogenicity in autaptic hippocampal neurons. (**a**) Average traces of glutamatergic synaptic responses induced by sucrose solutions from a subset of *Cplx*-TKO neurons (500 mM, n = 33; 250 mM, n = 19) and *Cplx*-TKO neurons expressing WT *Drosophila* Complexin (dmCplx) (500 mM, n = 35; 250 mM, n = 16). (**b,c**) Peak release rates of 500 mM (**b**) and 250 mM (**c**) sucrose solution-induced transmitter release. (**d**) Fraction of RRP released by 250 mM sucrose solution. Data are expressed as mean ± SEM; ***, $P < 0.0001$ compared to *Cplx*-TKO neurons. The numbers of neurons analyzed are indicated on the bars.

Supplementary Methods

Virus production and infection

The pSFV1 vectors containing CplxI variants together with the helper vector (pSFV-Helper 2, Invitrogen) were used to produce SFV particles. Viral and helper vectors were linearized with restriction endonuclease Spe I (New England Biolabs) and transcribed *in vitro* with capped RNA transcription kit mMESSAGE mMACHINE SP6 (Applied Biosystems). The RNAs were electroporated into BHK-21 cells and viral supernatants were harvested 24 hours after electroporaton. Viruses were titered with BHK-21 cells and about 1–3 × 10^6 infectious virus particles were used to infect cultured hippocampal neurons in a 35 mm–diameter well with 2 ml culture medium 9–12 hours before the experiments. Under this condition, the viral expression levels of CplxI variants are about 4 to 7-fold of the endogenous CplxI level of *CplxII*-KO neurons (**Supplementary Fig. 1**).

Lentiviruses were produced by co-transfecting HEK 293T cells with the lentiviral vector and two helper vectors, pVSVg and pCMV-delta R8.9 (ref. [1]). Viral supernatants were collected 48–72 hours after transfection and virus particles were concentrated using a centrifugal filter device (Amicon Ultra-15, Millipore). Viruses were titered with wildtype hippocampal mass-cultured neurons and about 2.3 × 10^6 infectious virus particles were used to infect neurons in a 35 mm-diameter well containing 2 ml culture medium within 24 hours after plating neurons. Under this condition, the viral expression level of dmCplx is about 2-fold of the endogenous CplxI level of *CplxII*-KO neurons[2].

Electrophysiology of cultured neurons

Action potential-evoked EPSC was triggered by a 2 ms somatic depolarization to 0 mV. Neurons were stimulated at 0.2 Hz in standard extracellular solution to measure basal EPSCs. 500 mM sucrose solution was directly applied onto the neuron for 4 s (hippocampal glutamatergic neurons) or 5 s (striatal GABAergic neurons) to measure readily releasable vesicle pool (RRP) size. For the experiments with dmCplx, 500 mM sucrose solution was applied for 8 s to ensure the depletion of RRP because dmCplx slows down the release (**Supplementary Fig. 6**). RRP size was determined by the charge transfer of the transient synaptic current induced by sucrose solution. Evoked EPSC was integrated for 1 s to calculate the charge transfer. Vesicular release probability (P_{vr}) was calculated by the ratio of evoked EPSC charge and RRP size. Short-term plasticity was examined by evoking synaptic responses at 50 Hz in standard external solution. Paired-pulse ratio was measured by dividing the second EPSC amplitude with the first EPSC amplitude. To examine the apparent Ca^{2+}-sensitivity of release, EPSCs were evoked at 0.2 Hz in 12 or 1 mM Ca^{2+}-containing solution. Each test measurement was preceded and followed by a measurement in standard extracellular solution to control for the rundown of synaptic responses. The EPSC amplitude at 12 or 1 mM Ca^{2+} concentration was normalized to the amplitude in standard extracellular solution. mEPSCs were recorded for 1–2 minutes in the absence and presence of a glutamate receptor antagonist, kynurenic acid (3 mM). Recordings in the presence of kynurenic acid were used to subtract the false positive events. To detect mEPSC events, traces were digitally filtered at 1 kHz offline and events were automatically selected with a scaled-template algorithm[3] in AxoGraph X. The template function is a double exponential with a scalable amplitude,

a rise time constant of 0.5 ms, a decay time constant of 4 ms, a baseline of 5 ms, and a template length of 10 ms. False positive events were subtracted as described[4].

Supplementary References

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