

Expanded View Figures

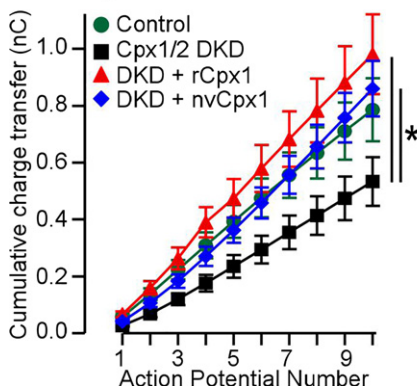
Comparison of mouse and *Nematostella* complexin sequences

mCpx1	MEFV MKQALGGATKDMGKMLGGDEEK -----DPDAAK KEEER -QEAL RQAEERKAKYAKMEAE REVMRQGI RDKYGI	72
mCpx2	MDFV MKQALGGATKDMGKMLGGEEK -----DPDAQ KKEEER -QEAL RQEEERKAKHARMEAE REKVRQOI RDKYGL	72
mCpx3	MAF VMKSMVGGQLKNI TGSLGGGEDKGDGDS-AAEAQ GMSREYEEYQKQLV EEEKMERDAQ FTQ RKAERATL RS HFRDKYRL	82
mCpx4	MA FFVKNMIS NQV KNLG FGGG GSEKKEEG TS DPAAAKGM TREY EEYQKQMI EEEKMERDA AF TQ KAERAC LRVHL RD KYRL	83
nvCpx1	MN PLTKALV TN KLSSV TKSI GLDDK DETTSE-----DAGV SSKEMR KMRE KEEAERAK REEM YAKRNAD REKK REQMRA KYGI	77
nvCpx2	MA SFAAKYL VS SATGK VQ STVGE FT R DSS-----NDG FKKEE LE KAEQQLQ KDEQ ERKKK FA KLAKR SKHRE KILNKYGI	74
mCpx-1	KK KEERE A EAQVAMEAN SEGL TRPKKAI PPG CGDEPEE DE SILDT VIK YLP GPLQ DMF KK*	134
mCpx-2	KK KEEKE A EKAAL EQ CEGS L TRPKKAI PAG CGDEE EEEE SILDT VLK YLP GPLQ DMF KK*	134
mCpx-3	PK NETDES Q IQLAG ---D VELPRE LAK MI EEDT- EEEE D KASV L GQ-LAS LPGLD LSSLK DKA Q TT LGD L KQSAE -K CHIM *	158
mCpx-4	PK SEMDET Q IQLAG D---D VDLP EDL RKMV DEDQ- DEEE E KD S ILGQ-LQ NLQ NMD LDT IK EKA Q AT FT E IKQSAE Q KCSVM *	159
nvCpx1	Q KDK-D GP PKSGG HK-- EEG S DAP TR KGSL NRE-- KSSE ED DN -----K CAIM *	121
nvCpx2	E KSKR HE PMETH SAA-- KML S IRGN SEER DYL-L DE EE DD GC S PCT NC T CF PLRSK T KIV *	135

Figure EV1. Alignment of mouse and *Nematostella* complexin sequences.

Alignment of complexin sequences from mouse (mCpx1–4) and *Nematostella vectoriensis* (nvCpx1 and 2). Sequences are shown in single-letter amino acid code; residues shared by 6, 5, or 4 of the 6 sequences are highlighted in yellow, green, and blue, respectively. Conserved hydrophobic sequences are highlighted in red, and C-terminal cysteine residues that are presumably isoprenylated are displayed on a black background.

A Cumulative charge transfer during 10 Hz stimulus trains



B Normalized synchronous EPSCs during 10 Hz stimulus trains

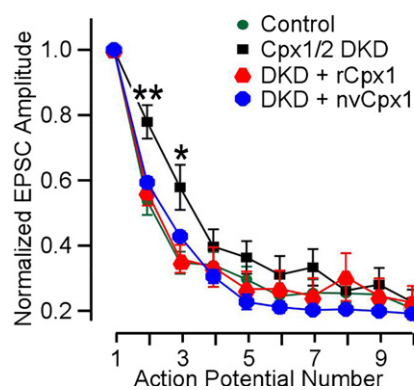


Figure EV2. Analysis of the kinetics of evoked NMDA receptor-dependent EPSCs (related to Fig 4E).

A The plot of cumulative charge transfer during the 10-Hz stimulus train as a function of the action potential number is recorded in WT cortical neurons that were infected with a control lentivirus (Control) or a lentivirus expressing complexin shRNAs (Cpx1/2 DKD) without or with co-expression of rCpx1 or nvCpx1.

B The degree of synaptic depression as a function of the action potential number is plotted as described for (A).

Data information: Data shown are means \pm SEM; statistical assessments were performed by two-way ANOVA (A) or Student's *t*-test (B) comparing each condition to control ($*P < 0.05$; $**P < 0.01$). The data from the experiments shown in Fig 4E, and the number of neurons/cultures analyzed correspond to those listed in the legend to Fig 4E.

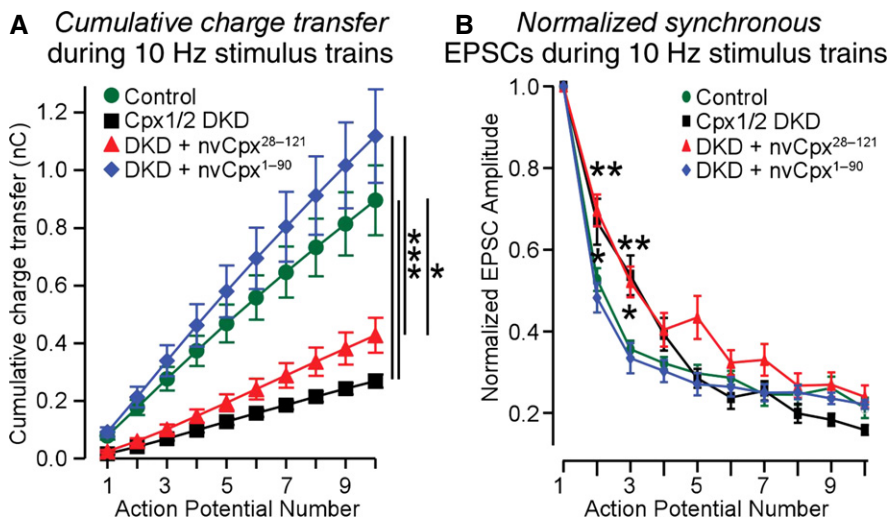


Figure EV3. Analysis of the kinetics of evoked NMDA receptor-dependent EPSCs (related to Fig 5E).

- A The plot of cumulative charge transfer during the 10-Hz stimulus train as a function of the action potential number is recorded in WT cortical neurons that were infected with a control lentivirus (Control) or a lentivirus expressing complexin shRNAs (Cpx1/2 DKD) without or with co-expression of nvCpx²⁸⁻¹²¹ or nvCpx¹⁻⁹⁰.
- B The degree of synaptic depression as a function of the action potential number is plotted as described for (A).

Data information: Data shown are means \pm SEM; statistical assessments were performed by two-way ANOVA (A) or Student's *t*-test (B) comparing each condition to control ($*P < 0.05$; $**P < 0.01$; $***P < 0.001$). The data from the experiments shown in Fig 5E, and the number of neurons/cultures analyzed correspond to those listed in the legend to Fig 5E.