

Supplemental Table 1. Summary for PTP and post-tetanic changes in the RRP size and P_r at the calyx of Held synapse under different pharmacological conditions. The estimates left and right to the arrow in each cell represent pre- and post-tetanic values, respectively (mean $\pm$ S.E.M.). A value in parentheses of each cell represents the relative percentile increase induced by tetanic stimulation. Superscript ‘i’ indicates that experiments were done at the synapse preloaded with a given drug. Drugs in *italic* were dissolved in DMSO (1/1000). Mean EPSC amplitude in the absence of AMPA receptor antagonists was 4.24 ± 0.67 nA (n=11). Abbreviations: KYN¹, 1 mM kynurenate; KYN², 2 mM kynurenate; γ -DGG, γ -D-glutamylglycine; TPP, tetraphenylphosphonium; CMZ, calmidazolium; MLCKip, MLCK inhibitory peptide-18; DMSO, dimethylsulfoxide; Che, chelerythrine; BIS, bisindolylmaleimide; CTZ, cyclothiazide.

Conditions		n	EPSC (nA)	RRP size (nA)	Release Probability
Control	KYN ¹	19	1.55 $\pm$ 0.15 $\rightarrow$ 2.97 $\pm$ 0.26 (100.33 $\pm$ 10.60)	4.32 $\pm$ 0.33 $\rightarrow$ 5.01 $\pm$ 0.37 (16.51 $\pm$ 1.20)	0.37 $\pm$ 0.03 $\rightarrow$ 0.60 $\pm$ 0.03 (70.57 $\pm$ 9.04)
	KYN ²	5	0.43 $\pm$ 0.07 $\rightarrow$ 0.99 $\pm$ 0.19 (128.56 $\pm$ 16.46)	1.98 $\pm$ 0.41 $\rightarrow$ 2.31 $\pm$ 0.42 (18.48 $\pm$ 2.83)	0.20 $\pm$ 0.02 $\rightarrow$ 0.42 $\pm$ 0.03 (107.20 $\pm$ 14.44)
	γ -DGG	11	0.88 $\pm$ 0.13 $\rightarrow$ 1.96 $\pm$ 0.27 (128.33 $\pm$ 14.75)	2.59 $\pm$ 0.19 $\rightarrow$ 3.09 $\pm$ 0.23 (19.25 $\pm$ 2.46)	0.34 $\pm$ 0.04 $\rightarrow$ 0.63 $\pm$ 0.06 (95.43 $\pm$ 13.42)
	γ -DGG (fura)	5	0.52 $\pm$ 0.15 $\rightarrow$ 1.13 $\pm$ 0.26 (131.35 $\pm$ 33.56)	3.05 $\pm$ 0.35 $\rightarrow$ 3.60 $\pm$ 0.39 (18.66 $\pm$ 2.44)	0.17 $\pm$ 0.04 $\rightarrow$ 0.33 $\pm$ 0.08 (94.26 $\pm$ 25.73)
TPP	KYN ¹	5	1.65 $\pm$ 0.29 $\rightarrow$ 2.44 $\pm$ 0.49 (46.44 $\pm$ 6.46)	5.93 $\pm$ 0.65 $\rightarrow$ 6.89 $\pm$ 0.75 (16.20 $\pm$ 1.97)	0.27 $\pm$ 0.03 $\rightarrow$ 0.35 $\pm$ 0.05 (26.54 $\pm$ 6.93)
	γ -DGG	6	0.84 $\pm$ 0.14 $\rightarrow$ 1.30 $\pm$ 0.23 (57.18 $\pm$ 13.27)	2.35 $\pm$ 0.20 $\rightarrow$ 2.71 $\pm$ 0.20 (15.90 $\pm$ 2.65)	0.32 $\pm$ 0.04 $\rightarrow$ 0.47 $\pm$ 0.05 (45.89 $\pm$ 7.73)
	γ -DGG (fura)	5	0.49 $\pm$ 0.15 $\rightarrow$ 0.72 $\pm$ 0.19 (53.44 $\pm$ 10.31)	2.81 $\pm$ 0.34 $\rightarrow$ 3.33 $\pm$ 0.36 (19.61 $\pm$ 3.19)	0.18 $\pm$ 0.04 $\rightarrow$ 0.22 $\pm$ 0.05 (26.92 $\pm$ 6.38)
Ru360 ⁱ	γ -DGG (fura)	6	0.43 $\pm$ 0.10 $\rightarrow$ 0.70 $\pm$ 0.19 (55.01 $\pm$ 9.34)	1.96 $\pm$ 0.44 $\rightarrow$ 2.27 $\pm$ 0.49 (16.66 $\pm$ 1.40)	0.25 $\pm$ 0.05 $\rightarrow$ 0.33 $\pm$ 0.07 (32.68 $\pm$ 7.25)
ML-7	KYN ¹	5	1.52 $\pm$ 0.30 $\rightarrow$ 2.48 $\pm$ 0.49 (71.25 $\pm$ 15.77)	4.10 $\pm$ 0.51 $\rightarrow$ 4.07 $\pm$ 0.58 (-1.59 $\pm$ 2.91)	0.36 $\pm$ 0.06 $\rightarrow$ 0.60 $\pm$ 0.08 (77.25 $\pm$ 13.16)
	γ -DGG	5	1.10 $\pm$ 0.24 $\rightarrow$ 1.95 $\pm$ 0.42 (79.45 $\pm$ 14.40)	3.52 $\pm$ 0.52 $\rightarrow$ 3.71 $\pm$ 0.64 (3.89 $\pm$ 2.55)	0.31 $\pm$ 0.08 $\rightarrow$ 0.53 $\pm$ 0.09 (80.64 $\pm$ 13.38)
CMZ	KYN ¹	5	1.78 $\pm$ 0.37 $\rightarrow$ 2.75 $\pm$ 0.47 (59.86 $\pm$ 12.69)	4.04 $\pm$ 0.59 $\rightarrow$ 4.26 $\pm$ 0.59 (5.72 $\pm$ 2.45)	0.44 $\pm$ 0.06 $\rightarrow$ 0.65 $\pm$ 0.06 (52.20 $\pm$ 10.98)
MLCKip ⁱ	KYN ¹	10	0.57 $\pm$ 0.09 $\rightarrow$ 0.91 $\pm$ 0.15 (64.89 $\pm$ 10.47)	2.64 $\pm$ 0.42 $\rightarrow$ 2.69 $\pm$ 0.47 (1.20 $\pm$ 1.66)	0.22 $\pm$ 0.02 $\rightarrow$ 0.35 $\pm$ 0.04 (62.24 $\pm$ 9.39)
MLCKip ⁱ & TPP	KYN ¹	7	0.64 $\pm$ 0.11 $\rightarrow$ 0.76 $\pm$ 0.12 (20.50 $\pm$ 7.37)	3.06 $\pm$ 0.43 $\rightarrow$ 3.09 $\pm$ 0.48 (-0.15 $\pm$ 3.42)	0.21 $\pm$ 0.02 $\rightarrow$ 0.25 $\pm$ 0.03 (21.35 $\pm$ 8.01)
DMSO _i	γ -DGG	5	0.85 $\pm$ 0.16 $\rightarrow$ 1.75 $\pm$ 0.31 (112.01 $\pm$ 15.90)	2.57 $\pm$ 0.32 $\rightarrow$ 3.16 $\pm$ 0.34 (23.96 $\pm$ 2.83)	0.33 $\pm$ 0.05 $\rightarrow$ 0.55 $\pm$ 0.06 (70.54 $\pm$ 10.01)
<i>Blebbistatin_i</i>	γ -DGG	6	0.35 $\pm$ 0.09 $\rightarrow$ 0.59 $\pm$ 0.13 (75.82 $\pm$ 10.35)	1.59 $\pm$ 0.10 $\rightarrow$ 1.60 $\pm$ 0.10 (0.35 $\pm$ 1.75)	0.21 $\pm$ 0.05 $\rightarrow$ 0.36 $\pm$ 0.07 (75.98 $\pm$ 12.39)
<i>Cheⁱ</i>	γ -DGG	6	0.59 $\pm$ 0.19 $\rightarrow$ 0.99 $\pm$ 0.26 (83.95 $\pm$ 15.06)	1.99 $\pm$ 0.37 $\rightarrow$ 2.39 $\pm$ 0.43 (20.20 $\pm$ 4.26)	0.31 $\pm$ 0.06 $\rightarrow$ 0.45 $\pm$ 0.09 (52.07 $\pm$ 6.74)
Ro31-8220 ⁱ	γ -DGG (fura)	4	0.31 $\pm$ 0.07 $\rightarrow$ 0.48 $\pm$ 0.11 (52.03 $\pm$ 4.88)	1.67 $\pm$ 0.35 $\rightarrow$ 1.62 $\pm$ 0.31 (-1.70 $\pm$ 2.44)	0.21 $\pm$ 0.06 $\rightarrow$ 0.33 $\pm$ 0.10 (54.76 $\pm$ 4.45)
<i>BIS-Iⁱ</i>	γ -DGG	5	0.81 $\pm$ 0.11 $\rightarrow$ 0.66 $\pm$ 0.08 (-17.26 $\pm$ 7.75)	2.30 $\pm$ 0.36 $\rightarrow$ 2.08 $\pm$ 0.40 (-10.96 $\pm$ 5.09)	0.38 $\pm$ 0.05 $\rightarrow$ 0.34 $\pm$ 0.05 (-7.26 $\pm$ 6.75)
<i>BIS-Vⁱ</i>	γ -DGG	4	0.90 $\pm$ 0.18 $\rightarrow$ 1.02 $\pm$ 0.14 (18.94 $\pm$ 11.52)	2.49 $\pm$ 0.22 $\rightarrow$ 2.39 $\pm$ 0.23 (-4.16 $\pm$ 2.77)	0.38 $\pm$ 0.08 $\rightarrow$ 0.45 $\pm$ 0.08 (24.20 $\pm$ 12.34)
CTZ	KYN ²	6	1.79 $\pm$ 0.24 $\rightarrow$ 3.57 $\pm$ 0.27 (110.46 $\pm$ 18.84)	6.12 $\pm$ 0.76 $\rightarrow$ 7.04 $\pm$ 0.80 (15.66 $\pm$ 1.73)	0.30 $\pm$ 0.03 $\rightarrow$ 0.52 $\pm$ 0.03 (81.62 $\pm$ 14.80)

Supplemental Fig. S1

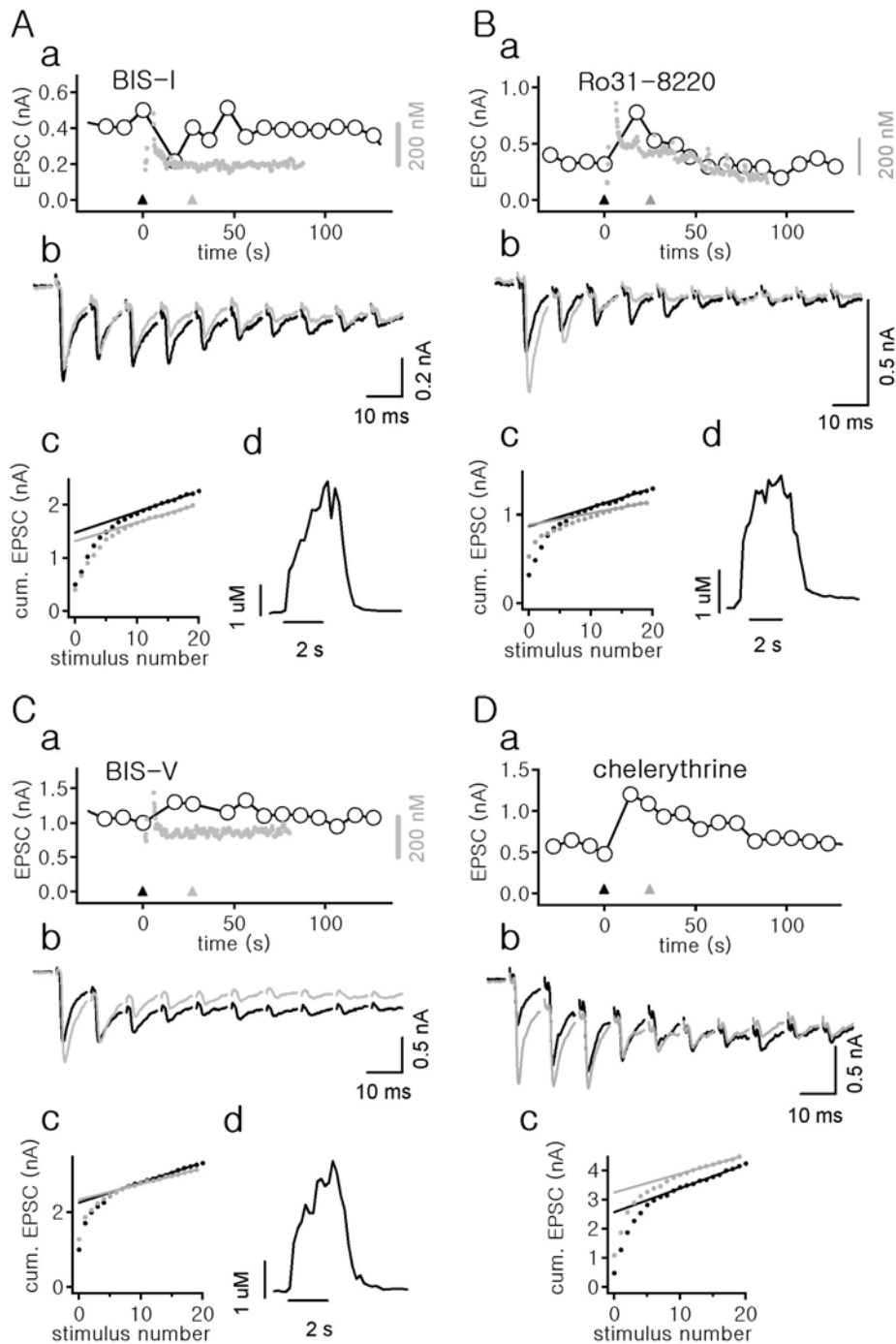


Figure S1. The effects of PKC inhibitors on PTP and Ca_{res} at the calyx of Held synapse. **Aa**, Representative time course of PTP and residual $\Delta[Ca^{2+}]_i$ (gray dots, scale bar) at the calyx of Held synapse preloaded with 2 μ M Bis-I in the presence of 2 μ M Bis-I and 4 mM γ -DGG in aCSF. **Ab**, First 10 EPSCs evoked by TS (100 Hz for 4 s) and by short AP trains 20 s after TS are superimposed. **Ac**, Plots of cum. EPSC amplitudes during (black) and 20 s after (gray) TS. Triangles in **Aa** indicate time points when TS (100 Hz, 4 s; black) or a short AP trains (100 Hz 20 APs; gray) was applied. **Ad**, Ca^{2+} transient evoked by TS. **B - D**, Similar experiments were repeated at the calyx of Held synapse preloaded with 2 μ M Ro 31-8220 (B), 2 μ M Bis-V (C) or 5 μ M chelerythrine (D).

Supplemental Fig. S2

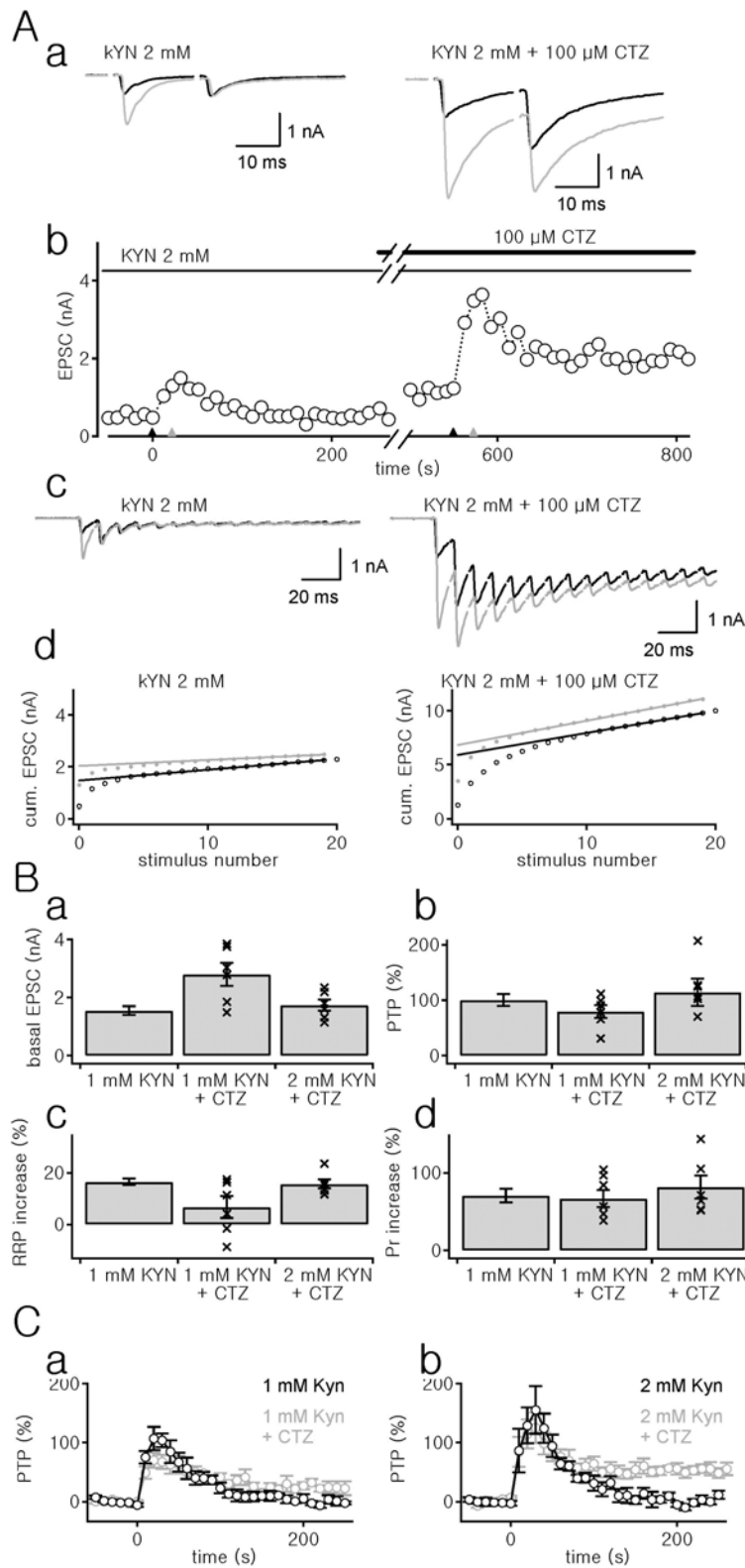


Figure S2. Effects of cyclothiazide (CTZ) on PTP. The basal EPSC was greatly enhanced in the presence of 1 mM Kyn plus 100 μ M CTZ, and the additional synaptic enhancement by PTP can saturate AMPA receptors. To test this possibility, we studied PTP in the presence of 2 mM Kyn plus 100 μ M CTZ. **Aa**, EPSCs before (black) and 30 s after TS (gray) in the presence of 2 mM Kyn (left) and after adding 100 μ M CTZ to the bath solution (right). **Ab**, Time courses of PTP induced by TS (100 Hz for 4 s) in the presence of 2 mM Kyn and after bath application of 2 mM Kyn plus 100 μ M CTZ. **Ac**, first fifteen EPSCs induced by TS (black) and by a short AP train (gray) applied 20 s after the TS are superimposed. **Ad**, Plots of cum. EPSC amplitudes during (black) and 20 s after (gray) TS are superimposed under control conditions (left) and after application of CTZ (right).

Triangles in Ab indicate time points when TS (*black*) or a short train of APs (20 APs at 100 Hz; *gray*) was applied. **Ba**, Mean values for basal EPSC were 1.6 ± 0.2 nA (n = 19), 2.8 ± 0.4 nA (n = 6) and 1.8 ± 0.2 nA (n = 6) in the presence of 1 mM Kyn, 1 mM Kyn plus 100 μ M CTZ or 2 mM Kyn plus 100 μ M CTZ, respectively. **Bb**, Mean values for PTP in the presence of 1 mM Kyn (reproduced from Fig. 1E.) or 1 mM Kyn plus 100 μ M CTZ ($79.7 \pm 11.7\%$) or 2 mM Kyn plus 100 μ M CTZ ($110.5 \pm 18.8\%$). **Bc**, Mean values for increases in the RRP size: $16.5 \pm 1.2\%$, $6.8 \pm 4.3\%$ (n = 6) and $15.7 \pm 1.7\%$ (n = 6). **Bd**, Mean values for the increase in P_r : $70.6 \pm 9.0\%$ (reproduced from Fig. 1E), $66.8 \pm 11.1\%$ (n = 6) and $81.6 \pm 14.8\%$ (n = 6). **Ca**, Averaged time courses of PTP in the presence of 1 mM Kyn before (*black*, n=5) after additional application of 100 μ M CTZ (*gray*, n = 5). **Cb**, Averaged time courses of PTP in the presence of 2 mM Kyn before (*black*) and after (*gray*) additional application of 100 μ M CTZ (n = 5).