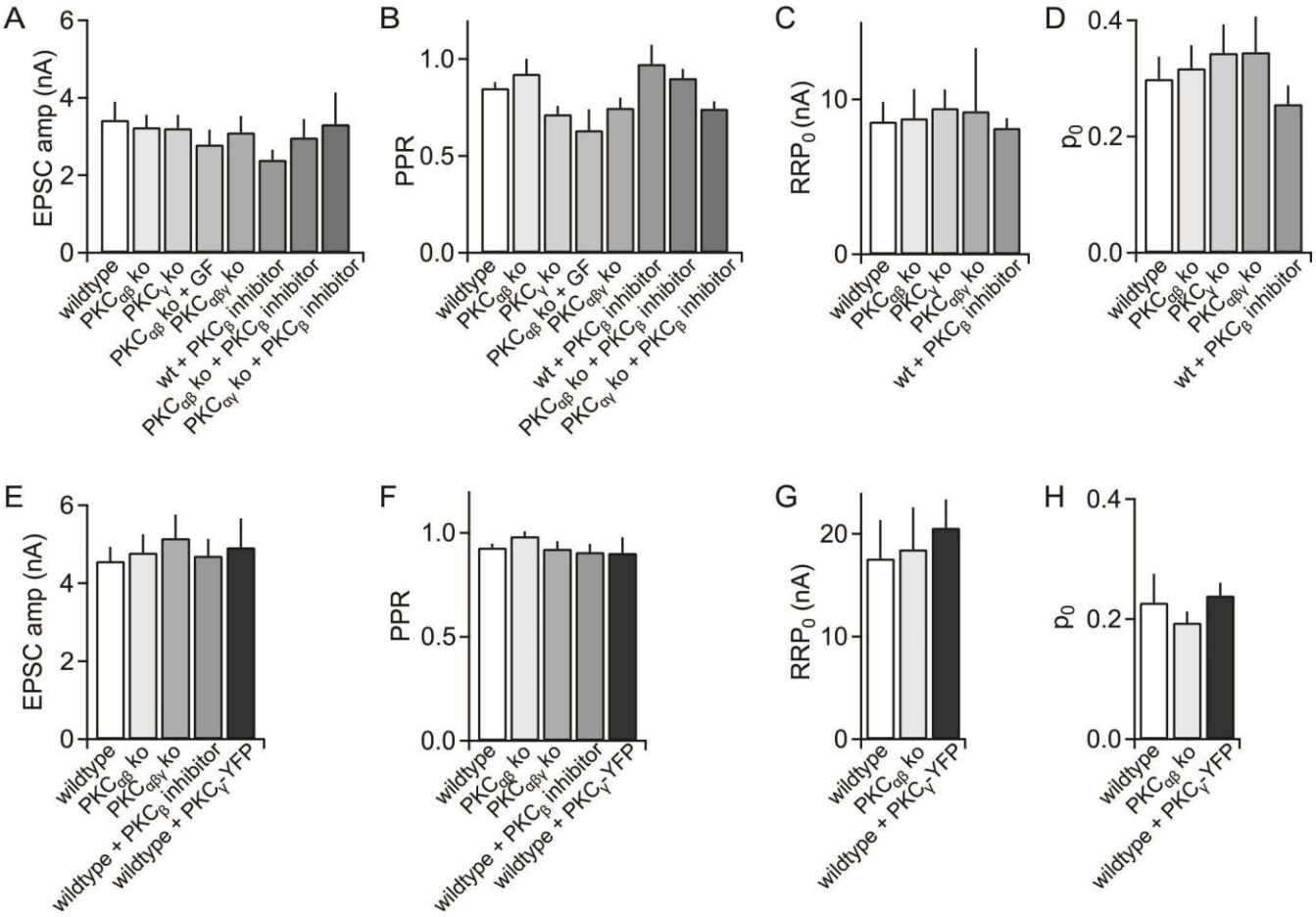

Figure S1. Basal properties of synaptic transmission for different knockout animals and in the presence of different PKC inhibitors for pre-hearing (A-D) and hearing (E-H) animals. (**A, E**) EPSC amplitude measured in standard extracellular solution at a holding potential of -60 mV. (**B, F**) The basal paired-pulse ratio (Δt = 20 ms) measured in all animal groups in standard extracellular solution. (**C, D, G, H**) Experiments performed in the presence of CTZ and kynurenate, as in **Figure 2**, were used to determine RRP_0 (**C, G**) and p_0 (**D, H**). Pre-hearing animals were P8-10 and hearing animals were P16-19, with the exception of the wildtype + PKCγ-YFP (P19-21). N=8-21 for each condition, bars are mean ± SEM, and there was no significant differences using one-way ANOVA tests in any of the parameters measured for each age group. Related to Figures 1, 2, 3, 5, 8 and 9.

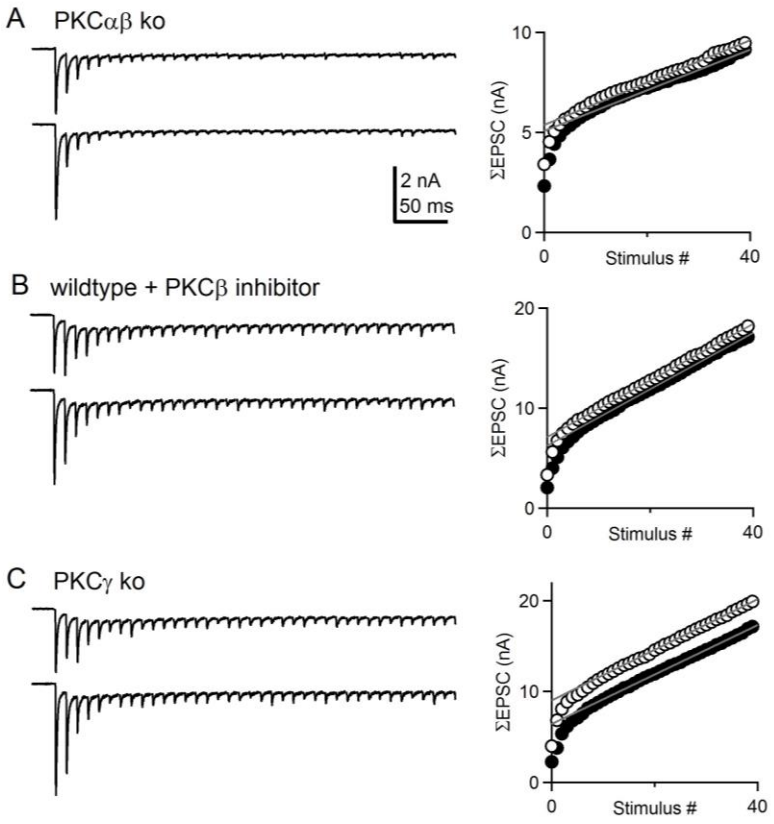

Figure S2. Estimating the contribution of RRP and *p* to PTP in calyces from pre-hearing animals. Representative experiments are shown that were used to assess the contributions of RRP and *p* to PTP in P8-10 animals for PKCαβ knockout animals (**A**), wildtype animals in the presence of a PKCβ inhibitor (**B**), and PKCγ knockout animals (**C**). Experiments were performed in the presence of CTZ and kynurenate to prevent AMPA receptor saturation and desensitization. Synaptic currents evoked by the first 40 stimuli of 4s, 100 Hz train (*top, left traces*) and by a 40 pulse 100 Hz train (*bottom, left traces*) 10 s after tetanic stimulation (at the peak of PTP) are shown. Graphs on the right show cumulative EPSCs plotted as a function of stimulus number. The linear fit (gray lines) to the last 15 points was back-extrapolated to the y-axis to calculate the ΣEPSC, which provides a measure of the RRP_{train}. Cumulative EPSCs for the first 40 stimuli of the tetanic train (closed circles) and for the second 40-pulse, 100 Hz train (open circles) are shown for representative cells from a PKCαβ knockout animals (**A**, *right*), wildtype animals in the presence of a PKCβ inhibitor (**B**, *right*), and PKCγ knockout animals (**C**, *right*). Related to Figures 3 and 5.

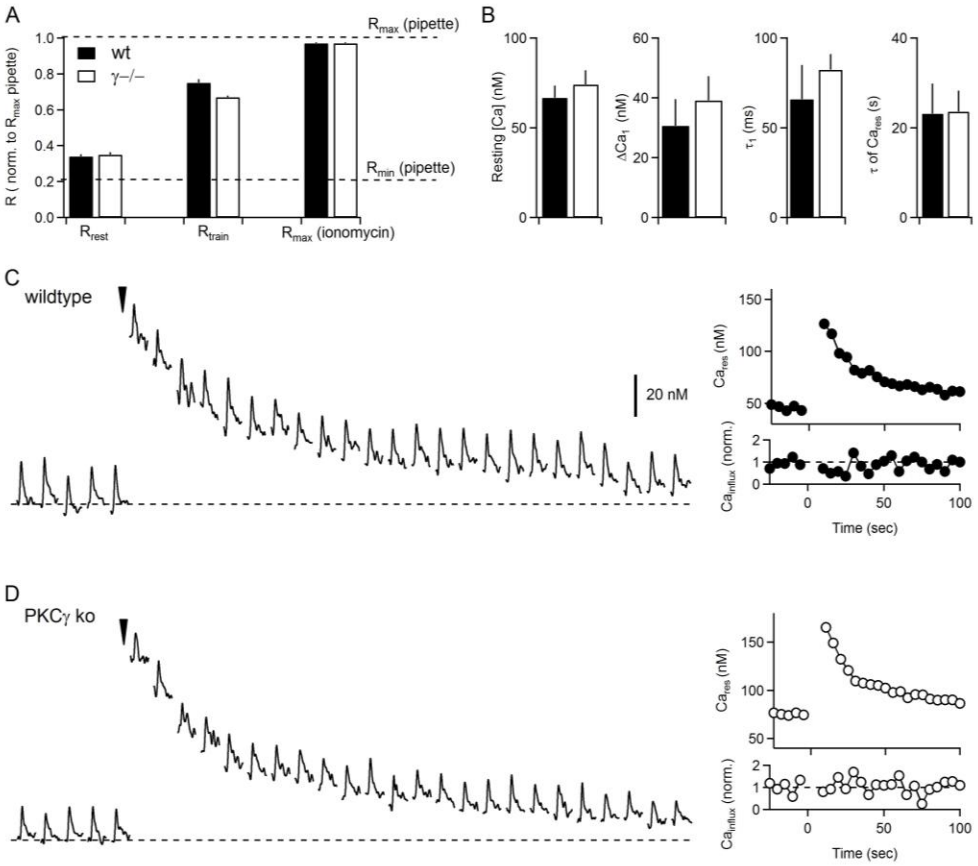

Figure S3. Presynaptic calcium measurements at the calyx of Held from pre-hearing animals. Calyces were bulk loaded with Calcium Green-1 dextran and Alexa 594. R is equal to the ratio of green to red fluorescence. (**A**) Calibration parameters are shown for calyces from P8-10 wildtype (wt) and PKCγ ko animals. R_{rest} is the ratio of a calyx measured in the absence of stimulation. R_{train} was the average ratio in a calyx (from 3.90 to 3.95 s after the onset of stimulation) measured during a 4 s, 100 Hz train and R_{max} ionomycin was measured in calyces following the bath application of ionomycin. Values are normalized to the maximum fluorescence (R_{max} pipette), which was obtained with a sealed pipette containing 10 mM $CaCl_2$. R_{min} was determined in a similar manner, but using a solution with 10 mM EGTA substituted for $CaCl_2$. (**B**) Comparison of the resting calcium concentration, the amplitude of calcium transients evoked by a single stimulus (ΔCa_1), the decay time constant of single calcium transients (τ_1), and the decay time constant (τ) of Ca_{res} following tetanic stimulation for wt and PKCγ ko animals. (**C**) Representative example showing calcium measurements from the P9 calyx from a wildtype animal. The calyx was stimulated at 0.2 Hz before and after tetanic stimulation (4 s, 100 Hz, ▼). Individual calcium transient (*left*) were measured for 512 ms with a 4.5 s gap between measurements, and for display purposes the plot does not reflect the time gap between calcium measurements. Plot of the Ca_{res} (*top, right*) and the normalized amplitude of the calcium transient (*bottom, right*) are shown. (**D**) Similar to **C** but for a calyx from a P10 PKCγ ko animal. Plots in (A,B) are mean ± SEM. Related to Figure 6.

Animal group (n)	PTP
P16-19	
Wildtype (10)	57 ± 11
PKCαβ ko (12)	8 ± 6
Wildtype + PKCβ inhibitor (7)	12 ± 1
P8-10	
Wildtype (12)	60 ± 10
PKCαβ ko (13)	40 ± 9
PKCγ ko (14)	54 ± 8
PKCαβγ ko (17)	12 ± 9
Wildtype + PKCβ inhibitor(10)	59 ± 15
PKCαγ ko (10)	61 ± 11
PKCαγ ko + PKCβ inhibitor (12)	17 ± 5
PKCαβ ko + PKCβ inhibitor (3)	54 ± 15
PKCαβ ko + pan-PKC inhibitor (11)	14 ± 3

Table S1. Magnitude of PTP. The number of cells (n) is listed next to each animal group for P8-10 animals (bottom) and P16-19 animals (top). Experiments were conducted in the absence of cyclothiazide and kynurenate. All values reported are mean percent changes ± SEM. Related to Figure 9.

Animal group (n)	PTP	RRP_{train}	p_{train}	RRP_{trainC}	p_{trainC}	RRP_{EQ}	p_{EQ}	PPR
P16-19								
Wildtype (8)	52 ± 9	40 ± 9	9 ± 5	34 ± 9	15 ± 6	30 ± 7	18 ± 5	-14 ± 5
PKCαβ ko (12)	19 ± 6	7 ± 5	17 ± 6	4 ± 4	21 ± 7	9 ± 5	17 ± 8	-20 ± 17
Wildtype + PKCγ-YFP (9)	75 ± 9	22 ± 6	63 ± 11	11 ± 5	58 ± 5	2 ± 4	74 ± 13	-35 ± 3
Wildtype + no PKCγ-YFP (3)	60 ± 7	52 ± 12	6 ± 7	45 ± 10	11 ± 7	25 ± 6	20 ± 5	-18 ± 1
P8-10								
Wildtype (10)	67 ± 16	21 ± 6	37 ± 11	18 ± 6	42 ± 12	11 ± 5	52 ± 15	-42 ± 8
PKCαβ ko (9)	42 ± 11	-0.4 ± 4	42 ± 7	-2 ± 4	45 ± 9	2 ± 3	45 ± 10	-35 ± 6
PKCγ ko (8)	64 ± 11	36 ± 12	21 ± 10	37 ± 13	20 ± 11	38 ± 13	17 ± 12	-12 ± 7
PKCαβγ (4)	7 ± 12	5 ± 7	6 ± 17	7 ± 10	5 ± 18	5 ± 6	4 ± 16	-6 ± 2
Wildtype + PKCαβ inhibitor (14)	56 ±17	9 ± 5	44 ± 16	8 ± 5	47 ± 16	9 ± 4	44 ± 15	-34 ± 5

Table S2. Contributions of RRP and *p* to PTP quantified using three different methods. Table summarizes the magnitude of PTP and the contributions of pool size (RRP) and release probability (*p*) for all animal groups. RRP and *p* data were calculated using the cumulative EPSC method (RRP_{train}, p_{train}), the corrected EPSC method (RRP_{trainC}, p_{trainC}) and the Elmqvist and Quastel method (RRP_{EQ}, p_{EQ}) for P8-10 animals (bottom) and P16-19 animals (top). The number of cells (n) is listed next to each animal group. These are different experiments than those summarized in **Table S1**, and all experiments were conducted in the presence of cyclothiazide and kynurenate. All values reported are mean percent changes ± SEM. Related to Figure 9.