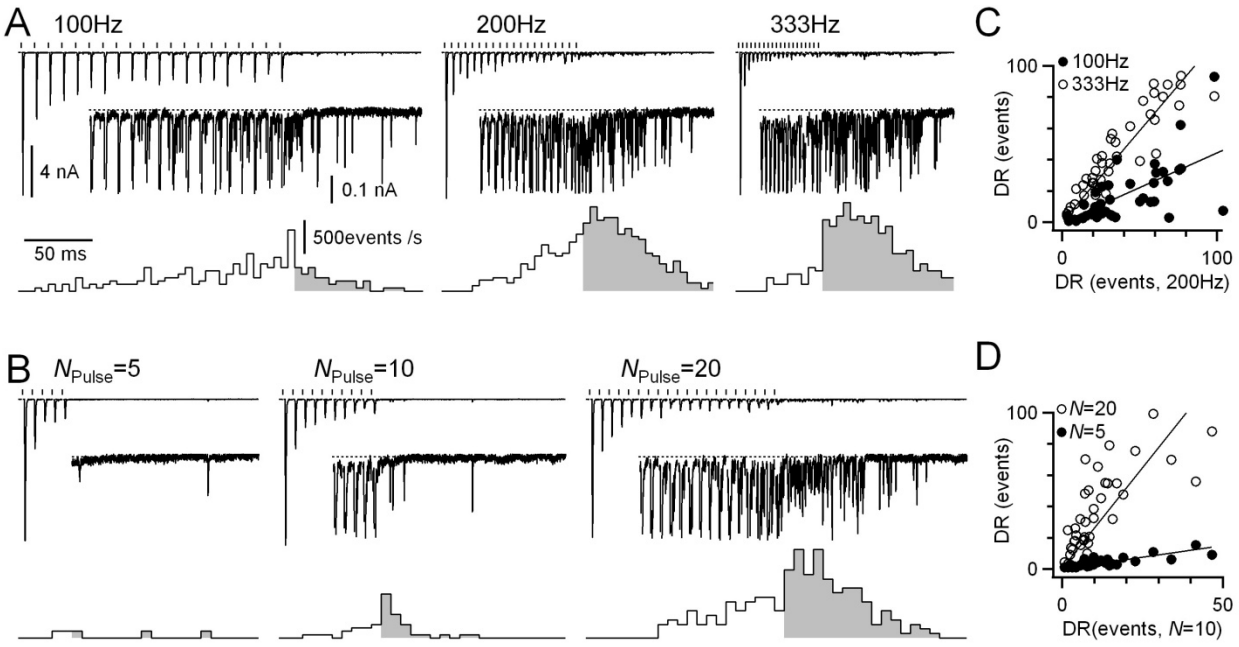
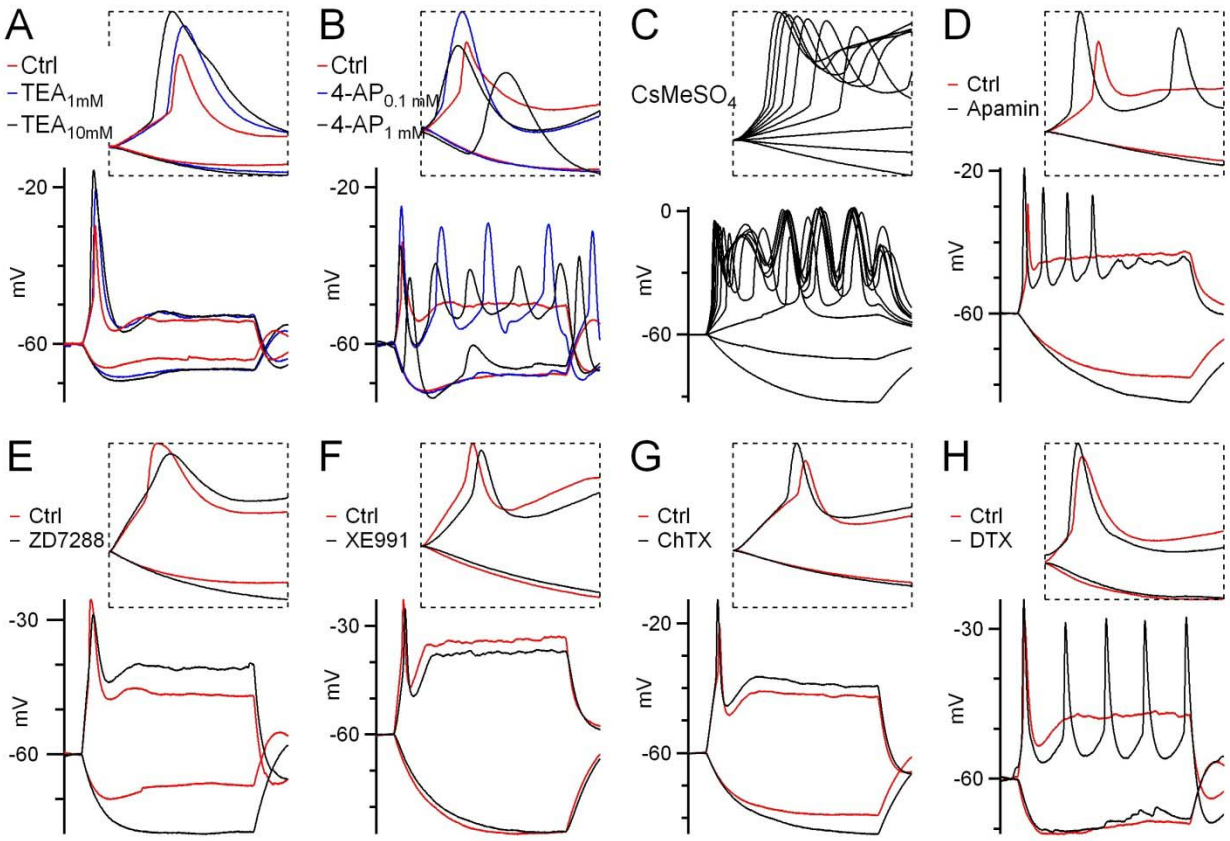
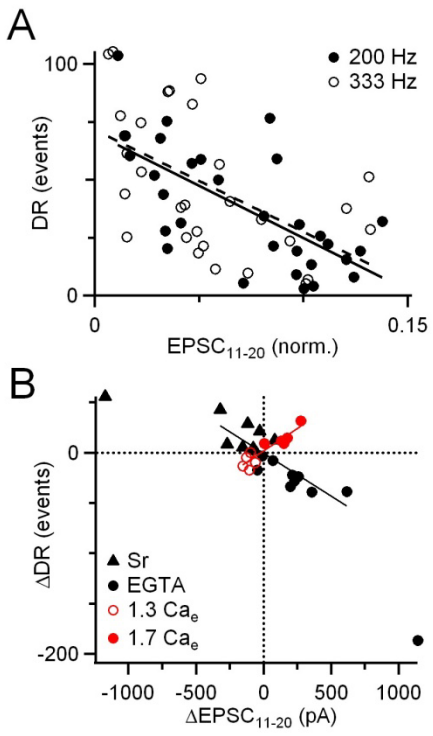


Supplementary Figure 1. Delayed release depends on frequency and intensity of stimulation. (A, B) Delayed release is enhanced with (A) higher frequency and (B) higher number of stimuli. Top traces show responses to 20-pulse trains at different frequencies (A), and 200-Hz trains of different lengths (B). Insets show magnified views of the traces, aligned in time, with the synchronous EPSC truncated. Lower histograms show the quantified delayed release rate. At the highest rates of delayed release, it was not possible to distinguish all events, particularly during the highest frequency trains (A, right).

(C, D) Levels of delayed release after the train (shaded regions in histograms of A and B) were strongly correlated for different frequencies (C, $N = 43$ endbulbs) or different numbers of stimuli (D, $N = 38$ endbulbs). In C, the best linear fit to the correlation between 100 Hz and 200 Hz had slope 0.44 ($r = 0.67$), and 1.17 for the correlation between 200 Hz and 333 Hz ($r = 0.92$). In D, the best linear fit had a slope of 0.30 between 5 pulses and 10 pulses ($r = 0.81$), and a slope of 2.58 between 10 pulses and 20 pulses ($r = 0.75$).

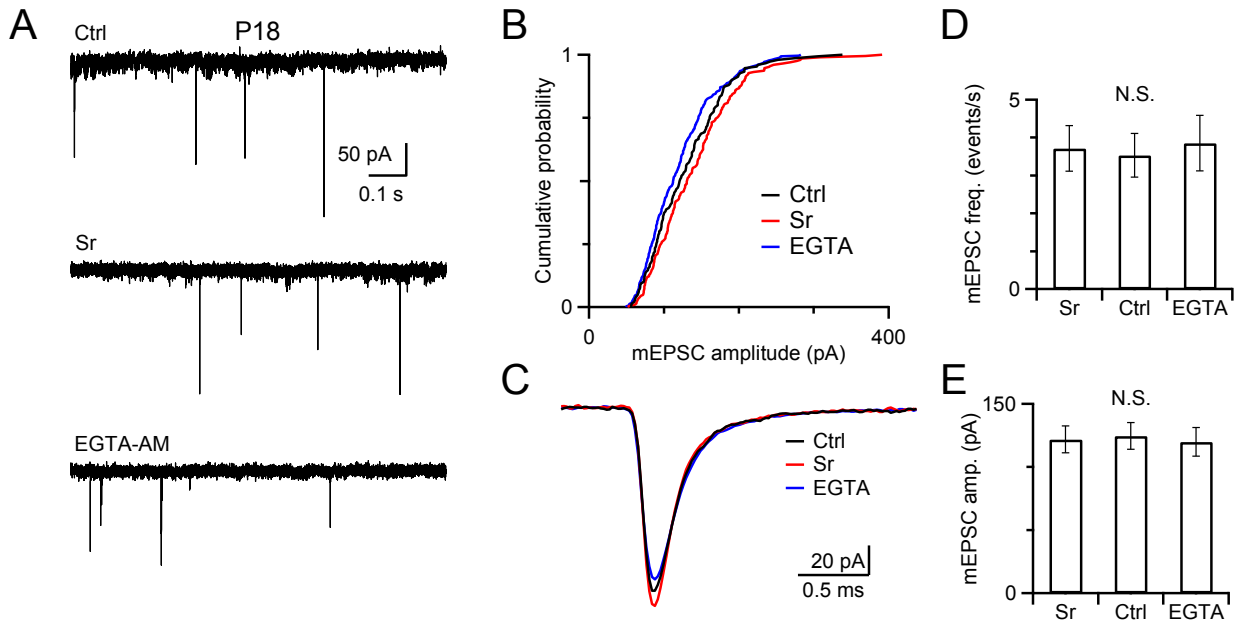


Supplementary Figure 2. Effect of different potassium channel blockers on spikes recorded in current clamp. Insets in dotted boxes show initial spike on an expanded scale. All examples are shown before (red) and after (black and blue) drug treatment, with the exception of Cs, which was present in the patch pipette, so no "before" is possible. We saw effects on spike width of TEA, 4-AP, apamin, and ZD7288. We saw effects on the sag potential for ZD7288. The sag potential was not present in all bushy cells. We saw effects on multiple firing for 4-AP, apamin, DTX, and Cs. 4-AP also led to considerable spontaneous firing.



Supplementary Figure 3. Levels of delayed and synchronous release are inversely correlated. (A) The number of delayed release events is inversely correlated with the amount of steady-state depression ($N = 31$ cells). Delayed release was counted over the first 400 ms after the train. Steady-state depression was quantified as the average EPSC amplitude over pulses 11–20 of a train normalized to $EPSC_1$. Correlation coefficients are $r = –0.66$ for 200 Hz and –0.54 for 333 Hz.

(B) Manipulations of delayed release using EGTA-AM and Sr cause changes in delayed release that are inversely proportional to changes in the steady-state EPSC ($r = -0.84$). Two outliers showing extreme effects are not included in the linear fit. Changing Ca_e leads to directly proportional changes in delayed release and steady-state EPSC ($r = 0.91$). These fits are significantly different (significant value for slope of fit $P < 0.005$, t-test).



Supplementary Figure 4. Treatment with Sr and EGTA-AM have no effects on mEPSC frequency or amplitude.

(A) Sample traces showing mEPSCs in one cell from a P18 animal in normal ACSF conditions (top), in the presence of 1 mM $SrCl_2$, and after treatment with 20 µM EGTA-AM for 5 min in normal ACSF.

(B) Cumulative distribution of mEPSC amplitude for the cell in A.

(C) Average mEPSC for the cell in A under different conditions.

(D, E) Effects of Sr and EGTA treatment in 9 experiments, showing mEPSC frequency (D) and average amplitude (E). There were no significant changes.