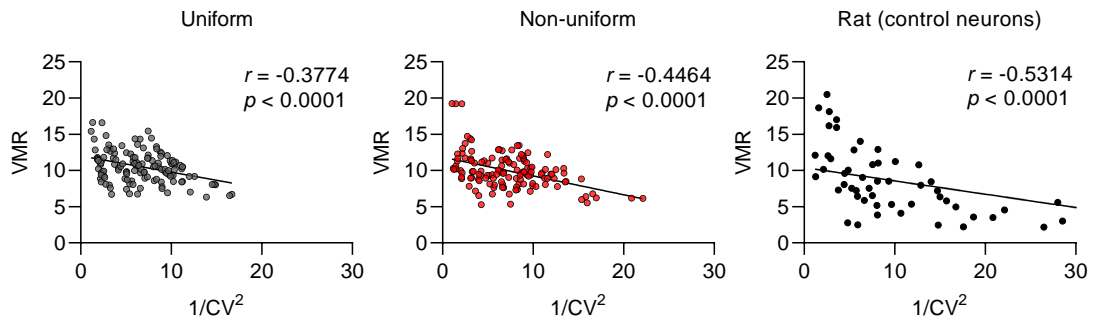
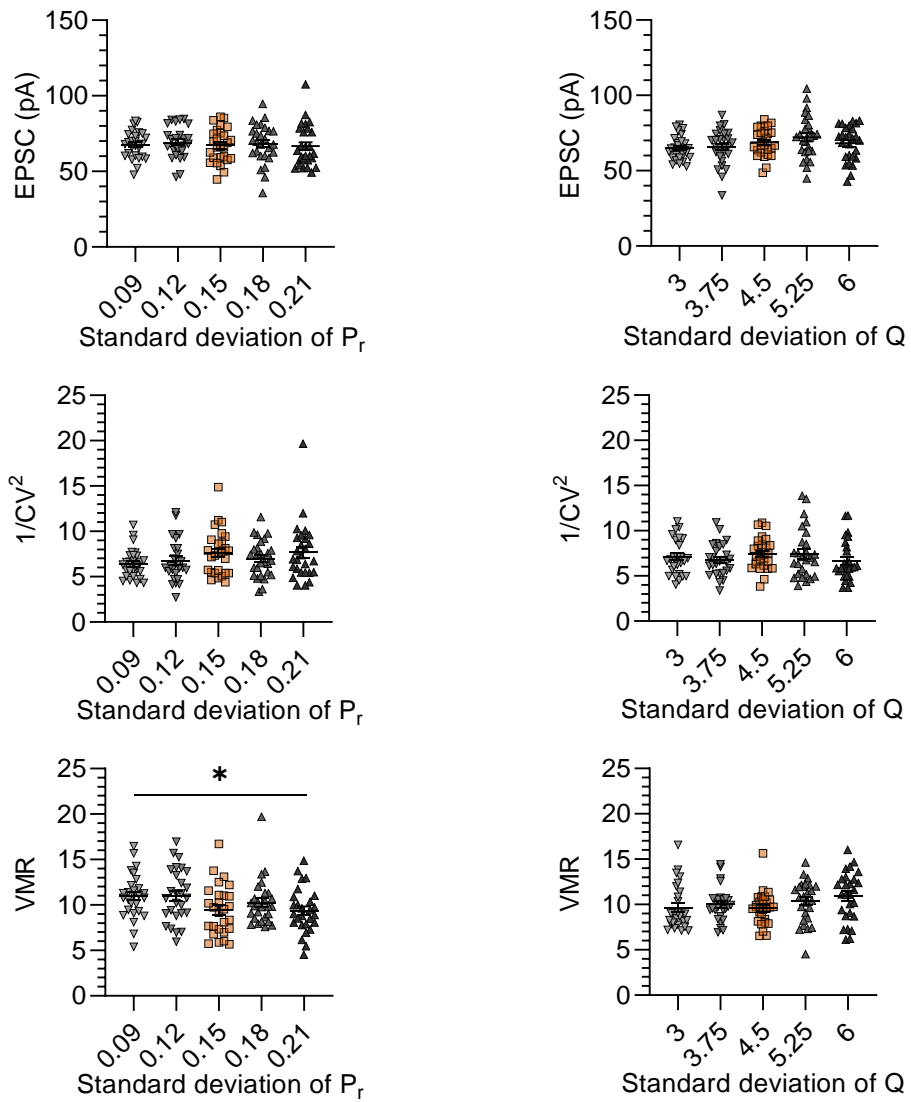


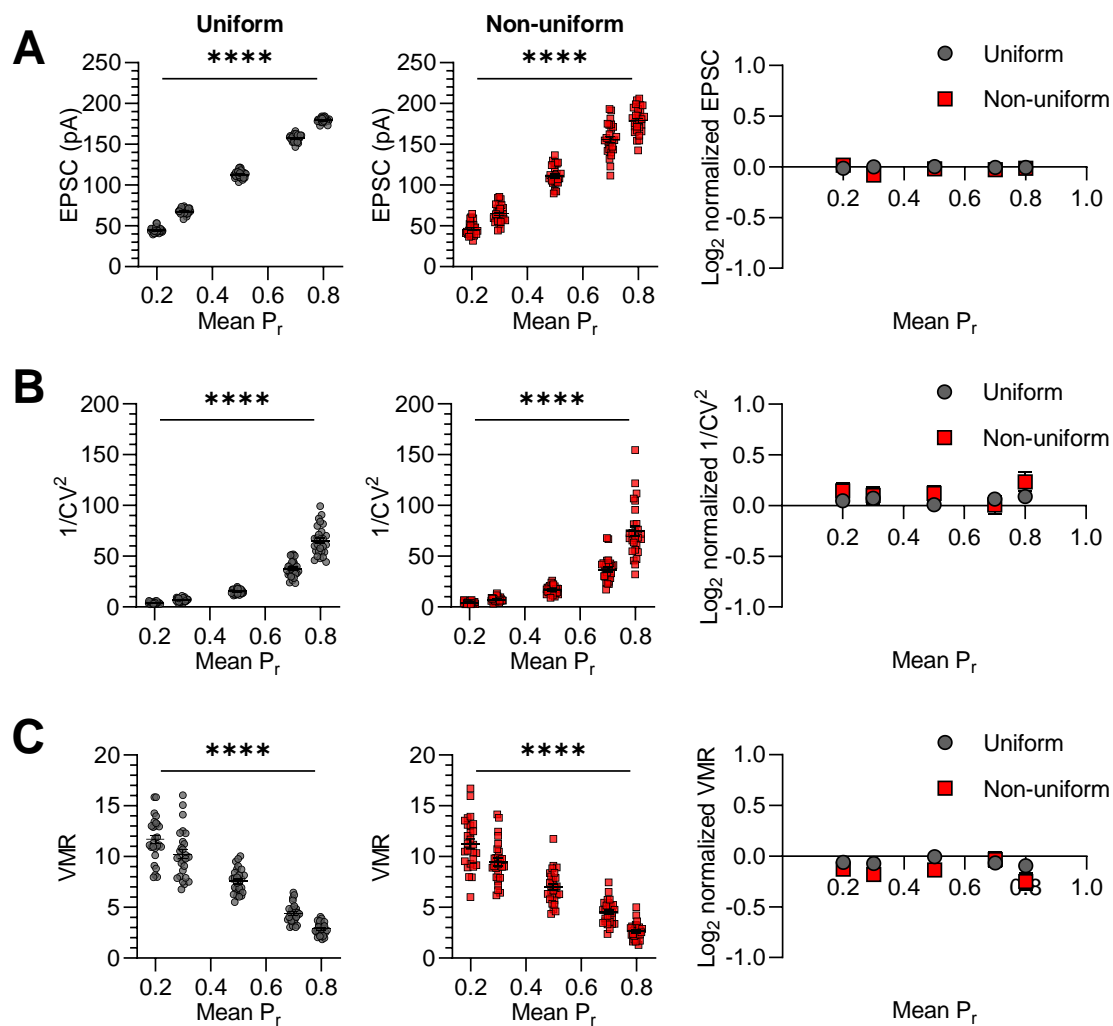
**SUPPLEMENTARY FIGURE S1.**  $P_r$  and  $Q$  for non-uniform populations are randomly drawn from physiological distributions. Examples of the randomly drawn values in a beta distribution with  $P_r = 0.3 \pm 0.15$  ( $\alpha = 2.5$ ;  $\beta = 5.8$ ) and a distribution in the Pearson system with  $Q = 15 \pm 4.5$  pA (skewness = 0.75; kurtosis = 4). In grey mean (solid line) and SDs (dashed lines) are depicted.



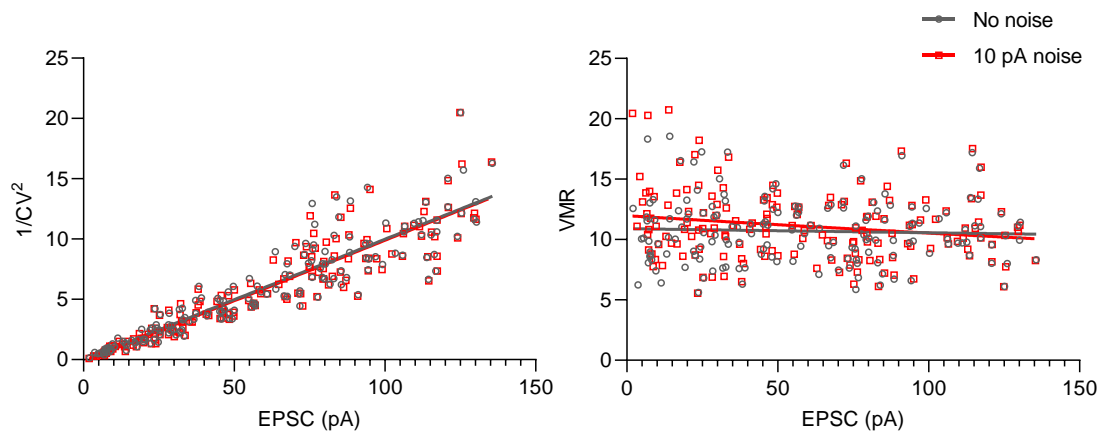
**SUPPLEMENTARY FIGURE S2.** In both the simulated and the electrophysiological data,  $1/CV^2$  and VMR show a negative correlation. Simulated neurons with uniform and non-uniform populations with  $N$  ranging from 5 to 25 (Figure 2) were used to compare  $1/CV^2$  and VMR values. For the electrophysiological rat data, untreated control neurons were used.



**SUPPLEMENTARY FIGURE S3.** Changing distributions of  $P_r$  and  $Q$  does not affect variance analysis outcomes. Effects of different SDs for  $P_r$  and  $Q$  on EPSC,  $1/CV^2$  and VMR.  $N$  (15), mean  $P_r$  (0.3) and mean  $Q$  (15 pA) were kept constant. Statistics: one-way ANOVAs were used to test effect of changing SDs. Error bars indicate SEM; \* $p < 0.05$ .



**SUPPLEMENTARY FIGURE S4.** Repetition of simulating changes in  $P_r$  and its effect on variance analysis outcomes (Figure 3). **(A-C)** Left panels: effects of changes in release probability ( $P_r$ ) on average EPSC **(A)**,  $1/CV^2$  **(B)** and VMR **(C)** in uniform (grey circles) and non-uniform (red squares) populations. Right panel: average  $\log_2$  values of EPSC **(A)**,  $1/CV^2$  **(B)** and VMR **(C)** normalized to expected values of uniform (grey circles) and non-uniform (red squares) populations. For all populations  $N = 15$  and  $Q = 15$  pA; in uniform populations SDs of  $P_r$  and  $Q$  were 0; in non-uniform populations SDs of  $P_r$  were 0.15 (0.14 for  $P_r = 0.2$  and 0.8) and SDs of  $Q$  were 4.5 pA ( $n = 27$ ). Statistics: effect of release probability on average EPSC,  $1/CV^2$  and VMR values (left panels) were tested using one-way ANOVAs. Normalized values were compared to 0 and between uniform and non-uniform using multiple  $t$ -tests with a Holm-Šidák correction. Error bars indicate SEM; \*\*\*\* $p < 0.0001$ .



**SUPPLEMENTARY FIGURE S5.** Intrinsic recording noise affects variance analysis outcomes at low EPSC amplitudes. Neurons with  $N$  values ranging from 2 to 25 (in 6 steps), while keeping  $P_r$  (0.3) and  $Q$  (15 pA) constant, were simulated ( $n = 162$ ; 6\*27 neurons). Noise was simulated by adding a random value between -5 and 5 pA to each sweep in each neuron. The EPSC values were compared with  $1/CV^2$  and VMR for neurons without noise (grey circles) and the same neurons, but with a random noise value added to their sweep EPSC values (red squares).