Supplemental Online Material

We estimated the size of the *RRP* in calyx and endbulb terminals from ΔC_m responses elicited by step depolarizations of up to 50 ms duration (Fig. 10). Our *RRP* estimates are considerably larger than those reported previously by Oleskevich et al. (2004). We therefore considered the possibility that our capacitance measurements may overestimate the *RRP* because many newly recruited vesicles contribute to the ΔC_m response. To this end, we modeled the effect of vesicle replenishment on our *RRP* estimates using published vesicle replenishment rates (see below). Assuming replenishment rates of 1/4 s^{-1} or 1/0.3 s^{-1}, the simple kinetic model fits the data plotted in Fig. 10B quite well and the deviation between model parameters and experimentally determined *RRP* size was only 0.8% and 13%. With a further increased, >25 times faster rate of replenishment (1/0.15 s^{-1}), the RRP was overestimated by only 25%. However, the deviation between experimental data (Fig. 10B) and predicted time course of vesicle depletion suggests that the mean replenishment rate is likely to be much lower in our experiments.

Taken together these simple numerical simulations suggest that even a >25 fold increase in the presumed replenishment rate from its basal level of 1/4 s^{-1} to 1/0.15 s^{-1} led to an overestimate of the *RRP* by a factor of only 1.25. In addition, it should be emphasized that vesicle endocytosis is entirely unaccounted for in the simple model described below because endocytosis proceeds usually on a time scale of seconds. If endocytosis were not negligible during the 60 ms, it would lead to an underestimation of the *RRP* and thereby counteract the effect of replenishment.

Simple kinetic model of vesicle release and replenishment

Let us assume here that a presynaptic terminal harbors a limited and fixed number of vesicle docking sites *N*. Each of these docking sites can exist in two states: (*i*) the occupied state (S_o, with a vesicle docked and ready for release) and (*ii*) the empty state (S_e, immediately after vesicle fusion). $N = S_o + S_e$ corresponds to the number of vesicles is the readily releasable pool ($N = RRP$). For simplicity, we assume that all docking sites are occupied at rest, i.e. $S_e = 0$ at $t = 0$. The transition between states S_o and S_e can be described by the following simple first order scheme:

$$S_e \underset{k_{release}}{\overset{k_{replenishment}}{\rightleftarrows}} S_o, N = S_e + S_o$$

$$\frac{\partial S_e}{\partial t} = -k_{replenishment} S_e + k_{release} S_o, S_e(0) = 0$$

$$\frac{\partial S_o}{\partial t} = k_{replenishment} S_e - k_{release} S_o, S_o(0) = N$$

Vesicle release, which is irreversible and proceeds only from the occupied state, can be described as follows:

$$S_o \xrightarrow{k_{release}} R$$

$$\frac{\partial R}{\partial t} = k_{release} S_o, R(0) = 0$$

The quantity measured in Fig.10B (ΔC_m) is proportional to the cumulative release of vesicles and thus represents an experimental estimate for R at time t. We can now solve this system of coupled differential equations numerically to estimated the time course of R under various conditions. In Fig. S1 we assume a fixed average rate of pool depletion of 1/0.01 s^{-1} (see Fig. 10B and also Hallermann *et al.*, 2003) and compare the results for three different (constant) replenishment rates: (*i*) a rate of 1/4 s^{-1} (Fig. S1A), (*ii*) a rate of 1/0.3 s^{-1} (Fig. S1B) and (*ii*) a rate of 1/0.15 s^{-1} (Fig. S1C). In each of the conditions we assume that the final ΔC_m measured after 60 ms should correspond to 1064 vesicles, i.e. the value estimated for endbulb terminals in Fig. 10B and then asked for the required N (=*RRP*) necessary to achieve this amount of exocytosis. The red curves represent the simulated time course of the cumulative vesicle release. Black symbols show experimental data.

Fig. S1A shows that for a slow rate of replenishment (1/4 s^{-1}) similar to that estimated from EPSC recordings at the calyx synapse (von Gersdorff *et al.*, 1997; Weis *et al.*, 1999; Billups *et al.*, 2005), the deviation between the cumulative release and the real N is only 0.8% (1064 vs. 1056 vesicles) because the number of newly recruited vesicles released during the 60 ms is negligible under these conditions. This simple kinetic model fits the data quite well.

Fig. S1B shows that for a >10 times faster rate of replenishment (1/0.3 s^{-1}), similar to that measured during strong elevations of $[Ca^{2+}]_i$ at the calyx of Held synapse (Sakaba & Neher, 2001; Hosoi *et al.*, 2007), *N* is overestimated by 13% (1064 vs. 945 vesicles). Since $[Ca^{2+}]_i$ is at resting level at the beginning of the presynaptic depolarization, the initial replenishment rate is expected to be significantly lower and therefore the number of newly recruited vesicles released during the 60 ms is likely to be overestimated in this scenario.

Fig. S1C shows that with a further increased, >25 times faster rate of replenishment (1/0.15 s^{-1}), *N* was overestimated by only 25% (1064 vs. 850 vesicles). Such a very rapid rate of recovery was estimated for a sub-fraction of vesicles (the *slowly releasing vesicles*) at the calyx of Held whereas the remainder of the *RRP* recovers much more slowly (Sakaba & Neher, 2001). It can be seen that such model does not fit the data well because all but the lowest measured ΔC_m values lie clearly above the curve predicted by the model.

References

Billups B, Graham BP, Wong AY & Forsythe ID. (2005). Unmasking group III metabotropic glutamate autoreceptor function at excitatory synapses in the rat CNS. *J Physiol* **565,** 885-896.

Hallermann S, Pawlu C, Jonas P & Heckmann M. (2003). A large pool of releasable vesicles in a cortical glutamatergic synapse. *Proc Natl Acad Sci U S A* **100,** 8975-8980.

Hosoi N, Sakaba T & Neher E. (2007). Quantitative analysis of calcium-dependent vesicle recruitment and its functional role at the calyx of Held synapse. *J Neurosci* **27,** 14286-14298.

Oleskevich S, Youssoufian M & Walmsley B. (2004). Presynaptic plasticity at two giant auditory synapses in normal and deaf mice. *J Physiol* **560,** 709-719.

Sakaba T & Neher E. (2001). Calmodulin mediates rapid recruitment of fast-releasing synaptic vesicles at a calyx-type synapse. *Neuron* **32,** 1119-1131.

von Gersdorff H, Schneggenburger R, Weis S & Neher E. (1997). Presynaptic depression at a calyx synapse: the small contribution of metabotropic glutamate receptors. *J Neurosci* **17,** 8137-8146.

Weis S, Schneggenburger R & Neher E. (1999). Properties of a model of Ca^{++}-dependent vesicle pool dynamics and short term synaptic depression. *Biophys J* **77,** 2418-2429.

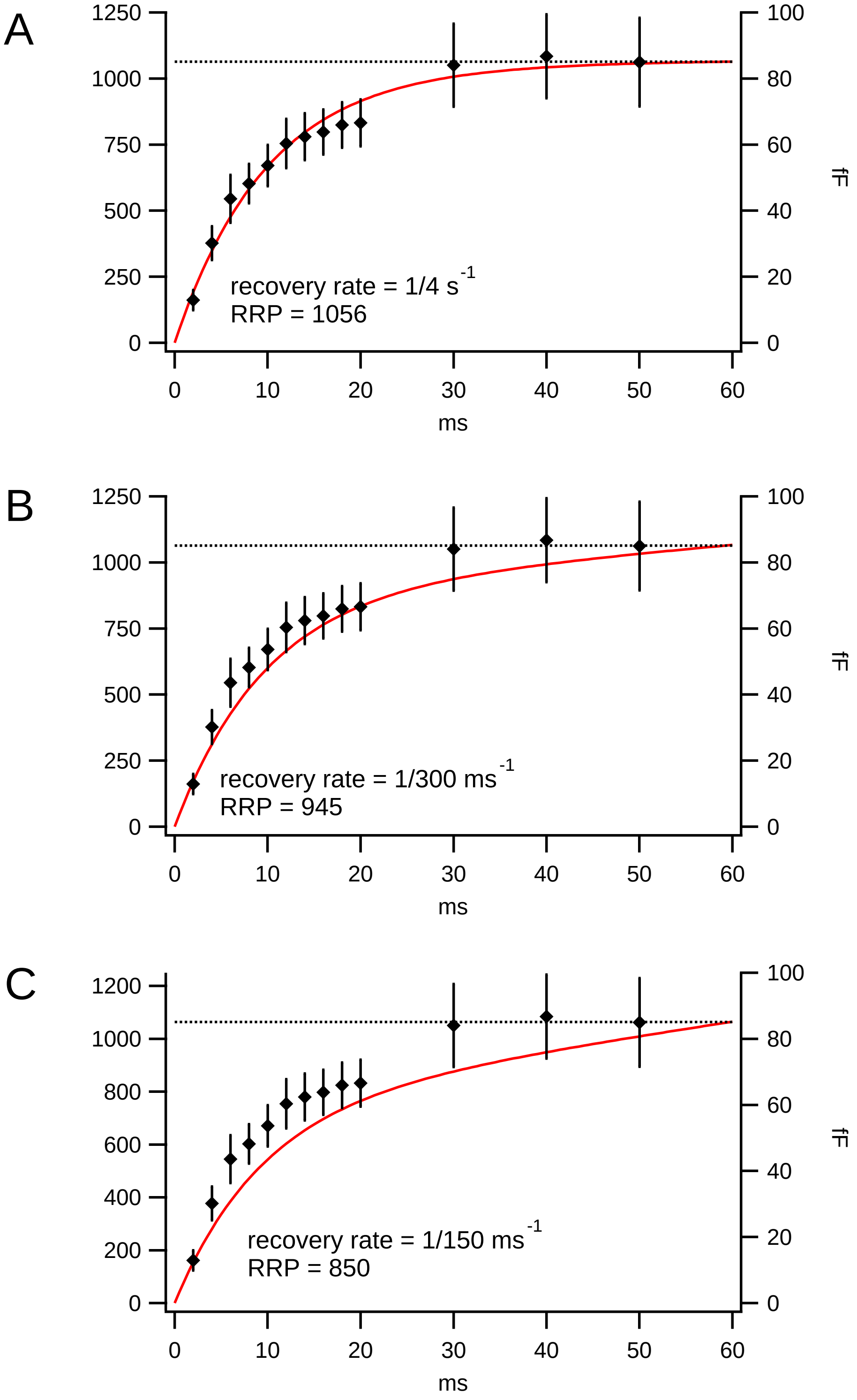
A
1250
1000
750
500
250
0
100
80
60
40
20
0
fF
recovery rate = 1/4 s-1
RRP = 1056
0
10
20
30
40
50
60
ms
B
1250
1000
750
500
250
0
100
80
60
40
20
0
fF
recovery rate = 1/300 ms-1
RRP = 945
0
10
20
30
40
50
60
ms
C
1200
1000
800
600
400
200
0
100
80
60
40
20
0
fF
recovery rate = 1/150 ms-1
RRP = 850
0
10
20
30
40
50
60
ms

Fig. S1