

Supplementary Information

Supplementary Figure 1. Brief electrical stimulation and hypertonic sucrose evoke comparable unloading of the RRP. **A**, Cultures were loaded with 10 μ M FM1-43 using either 200 action potentials (10 Hz) or 800 action potentials (80 Hz). Dye was washed away immediately after stimulation and cultures were left to rest for 10 min. The RRP was unloaded with either 60 action potentials (30 Hz) or application of 500 mM sucrose for 10 s. The reserve pool (RP) was unloaded with two 30 second applications of 50 mM KCl. **B**, Average proportion of the RRP and RP as a percentage of the SV recycling pool is shown (RRP - solid bars; RP – dotted bars). All experiments $n = 3$ (except 30 Hz unload of dye loaded at 10 Hz, $n = 4$) \pm SEM. No significant difference in the proportion of RRP unload was observed between electrical stimulation and sucrose stimulation (one-way ANOVA).

Supplementary Figure 2. Comparable unloading of the reserve SV pool using electrical stimulation and elevated KCl. **A**, Cultures were loaded with 10 μ M FM1-43 using either 200 action potentials (10 Hz) or 800 action potentials (80 Hz). Dye was washed away immediately after stimulation and cultures were left to rest for 10 min. The RRP was unloaded with 60 action potentials (30 Hz) and the reserve pool (RP) was unloaded with either three sequential trains of 400 action potentials (40 Hz) or two 30 second applications of 50 mM KCl. **B**, Average proportion of the RRP and RP as a percentage of the SV recycling pool is shown (RRP - solid bars; RP – dotted bars). All experiments $n = 4$ (10 Hz load) or $n = 3$ (80 Hz load) \pm SEM. No significant difference in the proportion of RP unload was observed between electrical stimulation and KCl stimulation (one-way ANOVA).



