



Figure 3-figure supplement 2: Different methods to estimate RRP size from HS responses. Red line represents a typical current response in a.u. induced by hypertonic stimulation. (A) HS induced current response is corrected for vesicle replenishment by taking the steady state current at the end of the response as baseline and subtracting this from the total current. Integration of the corrected current response yields the RRP size in nC, or in vesicles, after dividing total charge by the quantal content of a single mEPSC (green area) (14, 15). This gives an underestimation of the RRP since vesicle replenishment does not start at the maximal rate at the onset of the response but grows gradually during the stimulation. (B) RRP size is estimated from integration of the total charge transfer from the beginning of the response to an arbitrary timepoint after the peak (green area), neglecting any contribution from vesicle replenishment (grey area) (34, 40, 42, 43). This usually leads to an overestimation. (C) In this paper the definition of the steady state RRP in eq. (9) is used to infer the RRP size from the fitted model parameters. Effectively, in comparison to methods shown in A and B, we correct for vesicle replenishment by subtracting the calculated vesicle replenishment using eq. (20) (black line) from the total current. Integration of the corrected HS induced current response yields an accurate estimation of the RRP (green area).