Supplementary Figure 1. Standardizing the classification of mono- and multiphasic EPSCs

EPSCs were recorded at a sampling rate of 50 kHz with low pass filtering at 10 kHz. EPSCs were detected using a routine in Minianalysis, with a threshold set at 3 times the value of the root mean square of the baseline noise and subsequently accepted by eye.

To classify EPSCs as mono- or multiphasic, EPSC traces were transferred to Matlab (MathWorks) for further analysis with a custom written routine. A threshold of 20 pA was set for the detection of any signal above noise. The first derivative (in green) of the EPSCs (in blue) was calculated and low-pass filtered at 6 kHz. Firstly, the maximum of the EPSC was found, also marked as a zero crossing of the 1st derivative.

Monophasic EPSCs were defined as events with no inflections on rising or decaying phases (**A, B**). Multiphasic EPSCs were defined as EPSCs with inflections on rising and/or decaying phases (**C-F**). These inflections were interpreted as individual release events contributing to the multiphasic EPSC.

Detection of an inflection during the rising phase: If during the EPSC rising phase the 1st derivative showed a local maximum with a peak amplitude of >-300 pA/ms and was flanked by a change of > 90 pA/ms on either side of the local maximum, an inflection was scored as being detected. For example, in (**B),** the local maximum did not reach threshold (black dotted line), therefore the EPSC was classified as monophasic. However, in (**C**), the local maximum passed threshold and was flanked by a change > 90 pA/ms, an inflection was scored as being detected (black arrow) and therefore the EPSC was classified as multiphasic.

Detection of an inflection during the decaying phase: Slightly different criteria were used to detect an inflection during the decaying phase, as noise levels were higher and slopes less steep. If during the first 70% of the decaying phase the 1st derivative showed a local minimum <

225 pA/ms (red dotted line in (**D**)), an inflection was scored as being detected. In (**D**), inflections were detected in both the rising (black arrow) and the decaying phase (red arrow), the EPSC was classified as multiphasic.

E and **F** show examples of EPSCs (~ 3% of all EPSCs) that were missed by these criteria and had to be classified by eye as multiphasic EPSCs. The standardized procedure presented here using the 1st derivative and a post-sorting of questionable EPSCs by eye agreed well with the analysis completely done by eye. A subset of data was tested with both methods. 1 % of events classified by eye as monophasic were reclassified as multiphasic by the formalized approach; 4% of EPSCs classified by eye as multiphasic were reclassified as monophasic by the formalized approach.