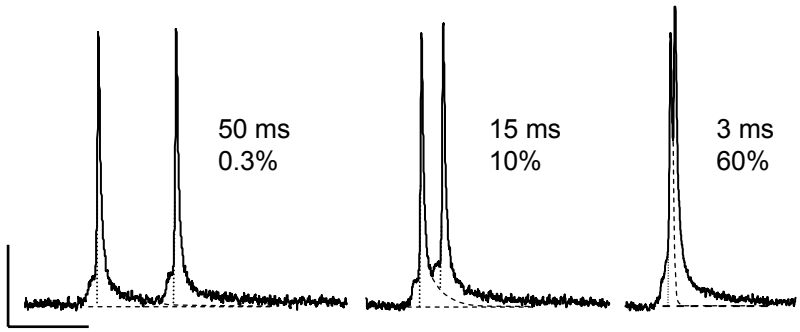

Supplementary Figure 3. Analysis of overlapping spikes

Any two amperometric spikes are considered overlapping if (i) the current at the end of the first spike is higher than at its beginning (uneven baseline), and (ii) the duration between the end of the first spike and beginning of the next is less then the average (among all spikes) $t_{1/2}$. The program first finds all amperometric spikes (see **Supplementary Methods**) and then checks for the overlaps. The baseline of the second overlapping spike is approximated using exponential fit constants of the falling phase of the first spike; if the fit is double-exponential, the slower τ is used. **Figure**. Overlaps of various degrees were generated by summating two identical recordings one of which was offset by a certain duration; the first and second spikes are therefore identical. Numbers next to the spikes indicate the offset and the extent of overlap. The latter was calculated as I_{saddle}/I_{max}*100, where I_{saddle} is the current at the minimum between the spikes and I_{max} is the amplitude of the first spike. Dashed lines represent spike baselines; dotted lines show PSF. Scale-bars are 50 pA and 50 ms. Data in the **Table** show % changes of the values for spike characteristics measured from overlapping spikes compared to those measured on the original singlet spike. Parameters that deviate from the true values by more than 15% are highlighted. Note that the differences between the original and the overlapping spike characteristics will be different when real spikes with unequal shapes are analyzed. Missing values for τ_2 indicate that spike's falling phase was fit with single exponential function.

Overlap	Spike	$t_{1/2}$ [ms]	I_{max} [pA]	Q [molecules]	t_{rise} [ms]	Rise [pA/ms]	Fall, τ_1 [ms]	Fall, τ_2 [ms]	I_{foot} [pA]	t_{foot} [ms]	Q_{foot} [molecules]
0.3 %	1	98	99	93	98	101	104	110	101	95	95
	2	100	100	107	98	103	98	104	112	90	101
5 %	1	99	99	87	101	98	**80**	91	96	92	89
	2	100	100	113	100	101	98	101	**81**	**63**	**42**
10 %	1	100	99	93	101	97	102	**81**	112	**75**	**84**
	2	97	97	107	99	97	98	112	**65**	**66**	**47**
25 %	1	107	106	85	101	106	99		**125**	**73**	93
	2	103	104	115	107	98	**117**	**155**	**61**	**9**	**8**
40 %	1	110	109	85	114	96	99		**125**	92	**116**
	2	100	105	115	115	94	**121**	**166**	**66**	**4**	**4**
60 %	1	**117**	109	**77**	109	102	**76**		**144**	90	**131**
	2	102	107	**123**	**133**	**87**	**123**	**170**	**42**	**1**	**1**