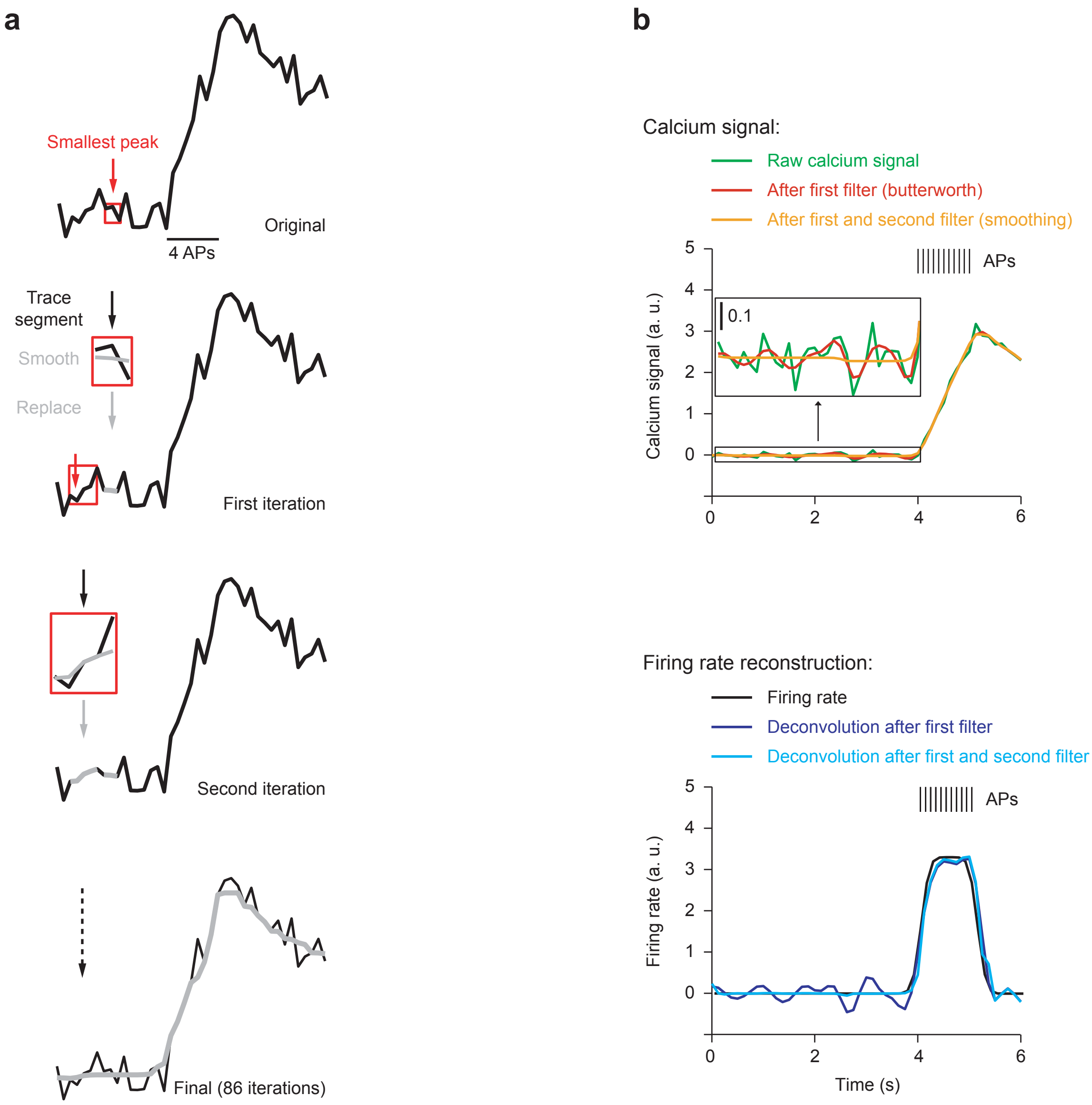

Supplementary Figure 3 | Smoothing of Ca^{2+} signals by iterative local averaging: principle and effect. (**a**) Illustration of iterative smoothing procedure. Black trace shows a simulated Ca^{2+} signal, generated by convolution of four discrete events (APs; 4 Hz; amplitude = 1) with an exponential kernel (τ = 3 s) and addition of gaussian noise (mean = 0; SD = 0.3). The iterative procedure first identifies the peak p_{min} with the smallest absolute amplitude, measured as the difference between the signal amplitude at each peak and the preceeding peak. Thr_{noise} was set to 1. If the peak amplitude is < thr_{noise}, a trace segment between the peaks adjacent to p_{min} is subjected to smoothing (black trace in red box). The value at each peak in the trace segment including the first and last data point is replaced by the average of the peak value and the neighboring values. This procedure is repeated three times. The original trace segment is then replaced by the smoothed segment (gray). In the next iteration, peak amplitudes are redetermined and the next smallest peak is smoothed by local averaging. This procedure is continued until all peaks with amplitudes smaller than thr_{noise} are removed, or until smoothing no longer changes the trace, or until a maximum number of iterations is reached (usually 5000). Bottom graph compares the final result (gray; reached after 86 iterations) to the original trace (black). Much of the noise is removed from the large peak without distortion of the rising flank. (**b**) Effect of iterative smoothing on deconvolution. Top: Green trace shows a simulated calcium signal, generated by convolving each AP (amplitude = 1) with an exponential kernel ($\tau = 3$ s) and addition of gaussian noise (mean = 0; SD = 0.15). 10 APs at a frequency of 10 Hz were delivered at t = 4 s (ticks). The calcium signal trace was first filtered with a low-pass butterworth filter (red). The filtered trace was then smoothed by the iterative smoothing procedure described in **a** (orange), which further reduced the noise (inset). Bottom graph compares the deconvolved calcium signal to the actual firing rate (black). When deconvolution is performed after the first filtering step (blue), the abrupt firing rate change is reconstructed well but the remaining noise results in baseline fluctuations. When the calcium signal is deconvolved after the second filtering step, fluctuations are substantially reduced without distortion of the reconstructed firing rate change. Hence, the iterative smoothing procedure can antagonize noise-related reconstruction artifacts without loss of temporal resolution. In practice, the effect of the iterative smoothing procedure was most pronounced when the signal-to-noise ratio was low, e. g., when temporal resolution was maximized.