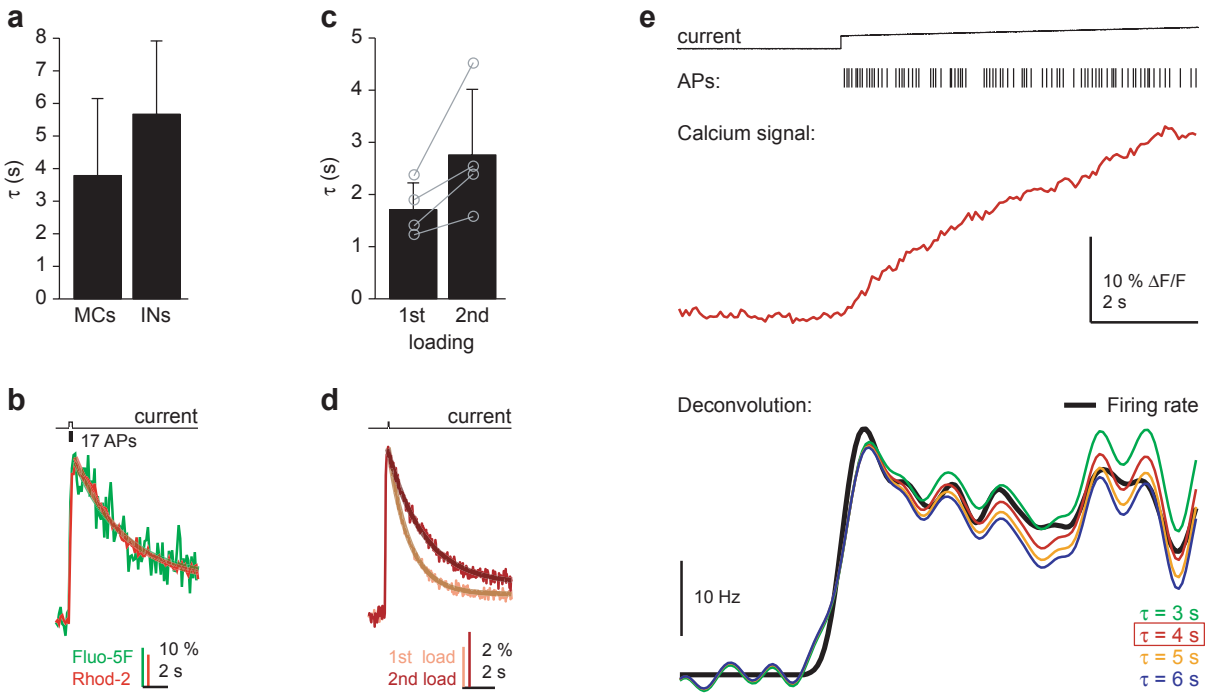

Supplementary Figure 2 | Decay time constants and their influence on deconvolution. (**a**) average decay time constants of Ca^{2+} transients measured with rhod-2 in MCs (τ = 3.8 ± 2.4 s; mean ± SD; n = 24) and INs (τ = 5.7 ± 2.3 s; n = 19). Some neurons were co-loaded with OGB-1 or fluo-5F. (**b**) Ca^{2+} transients in a MC loaded with rhod-2 (red) and fluo-5F (green) in response to a burst of 17 APs evoked by current injection. Thick lines are exponential fits to the decay phase with time constants of 3.9 s (red) and 4.0 s (green). (**c**) Dependence of decay time constant on indicator concentration. Superficial MCs were first loaded by a short focal pressure application of rhod-2-AM from a pipette just above the OB surface. This procedure produced very light staining. A recording was then established from an individual MC and the decay time constant of the Ca^{2+} transient was determined. The indicator concentration in the recorded neuron was then increased by a second pressure application of rhod-2-AM, and the time constant was measured again. In all 4 MCs tested, decay time constants increased after the second loading (lines; bars show mean ± SD). Moreover, time constants were lower than time constants of MCs after bolus loading inside the tissue, which produced more intense staining. These results indicate that the decay time constant depends on the intracellular indicator concentration, consistent with observations in dendrites of other neurons[1,2]. In the rodent neocortex in vivo, time constants of Ca^{2+} transients after bulk loading of OGB-1 are ~1 s[3]. One reason for the longer time constants observed in zebrafish neurons may be differences in indicator concentration. However, this cannot entirely explain the observed differences because time constants of zebrafish MCs were longer even when dye

loading was minimal. Hence, other factors, such as the difference in temperature, may also be involved. (**d**) Ca^{2+} transients evoked by a 50 ms current step in the same MC after the first and second brief loading of rhod-2-AM. Average over 8 repetitions. Thick lines are exponential fits to the decay phase with time constants of 1.4 s and 2.4 s. (**e**) Effect of kernel time constant, τ_{decay}, on firing rate reconstruction by deconvolution. Top: Sustained AP firing and associated Ca^{2+} signal evoked by a long current ramp in a MC. Bottom: Firing rate (black) and deconvolved Ca^{2+} signal using kernels with 4 different τ_{decay}. Best reconstruction was obtained when τ_{decay} matched the time constant of Ca^{2+} transients in the same neuron (4 s; red). However, other time constants yielded only modest deviations, particularly during the first 2 s of the response. Hence, a potential reconstruction error resulting from incorrect estimates of τ_{decay} is relatively small, even for sustained firing patterns.

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