Sensory-driven enhancement of calcium signals in individual Purkinje cell dendrites of awake mice

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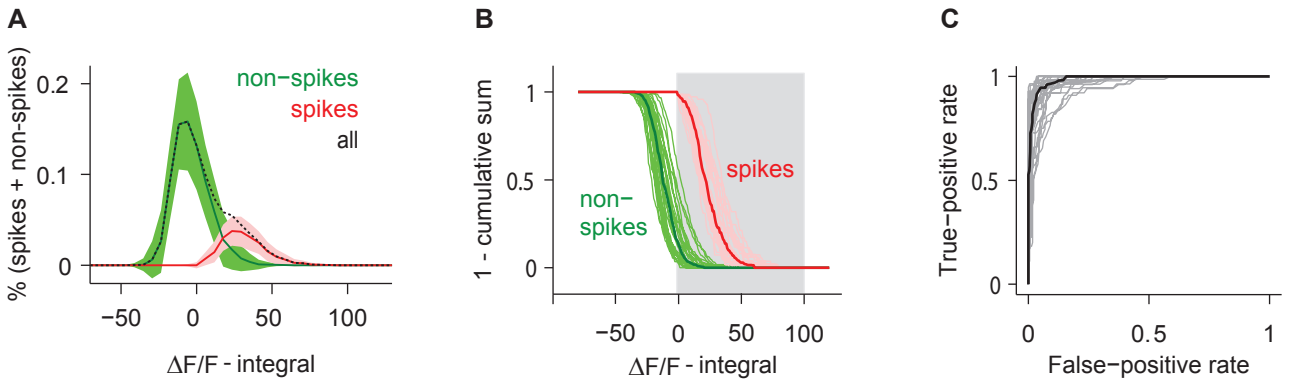

Figure S1, related to Experimental Procedures. Calcium spikes are largely separable from non-spikes based on their ΔF/F-integral value.

Calcium spikes were identified by our spike-detection algorithm, which involved template matching and threshold setting (see Experimental Procedures). In this figure we compare the ΔF/F-integral value of calcium spikes with non-spikes. Panel **A** shows the distribution of ΔF/F-integral values for calcium spikes (red), non-spikes (green), and the pooled data (dashed). Calcium spikes and non-spikes have largely distinct ΔF/F integrals (Figure S1**A**; mean±s.d.; bin width=6 ΔF/F.ms); however, there is a slight overlap between the two distributions. This overlap results from fluorescence signals that, despite having a rather large ΔF/F integral, were not identified as calcium spikes, because their temporal profile was different from the template used in our spike-identification algorithm. Figure S1**B** shows one minus the cumulative distributions of calcium spikes (red) and non-spikes (green) for all the dendrites (thick lines: median). These curves represent the rate of true positives (red) and false positives (green) in discriminating the spike, non-spike distributions based on the ΔF/F-integral values. In order to quantify the separation of the two distributions, we performed ROC analysis by plotting true-positive rates against false-positive rates for each dendrite (Figure S1**C**; black: median). The highly bowed ROC curves indicate that the distribution of ΔF/F integrals is largely separable for calcium spikes compared to non-spikes. Area under ROC curve, which represents the performance in discriminating spikes from non-spikes, equals 0.98±0.02, (mean±s.d.), demonstrating a very high discrimination rate.

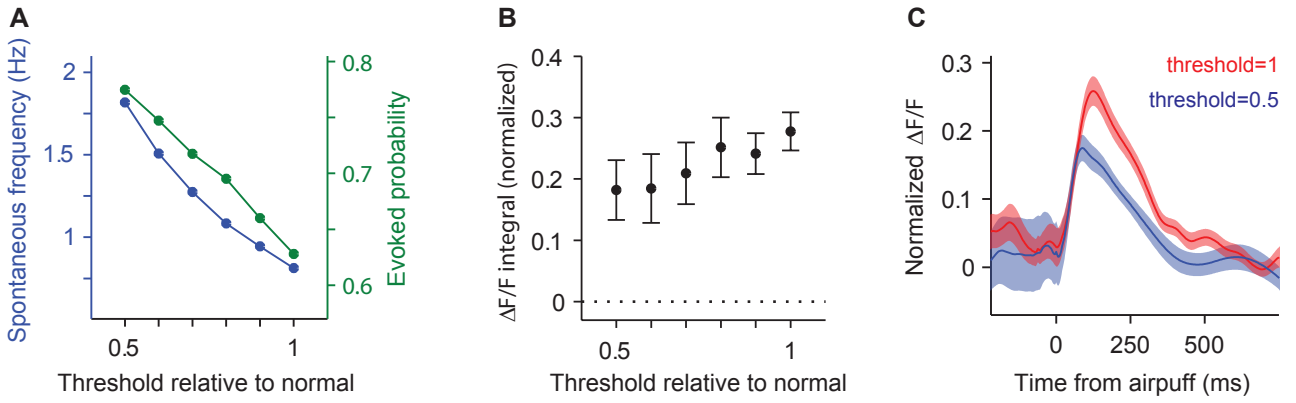

Figure S2, related to Figure 4. Non-CF signal cannot arise from missed calcium spikes.
Non-CF signal might represent small CF-triggered calcium spikes that we failed to identify. We addressed this possibility by changing the threshold of our spike-detection algorithm. As expected, the frequency of spontaneous calcium spikes (Figure S2**A**, blue) and the probability of airpuff-evoked calcium spikes (Figure S2**A**, green; bin width=100ms) were higher at lower thresholds (threshold=1 represents the normal threshold; dots represent averages across all dendrites; n=41). For instance when the threshold was changed to 50% of its normal value, the spontaneous frequency was approximately doubled (1.8 Hz at threshold 0.5 compared to 0.8 Hz at threshold 1). Since spontaneous calcium spikes happen at about 1 Hz, a frequency of 1.8 Hz suggests very few failures in detecting calcium spikes at threshold 0.5. Next we computed ΔF/F-integral for the non-CF signal at each value of the threshold (Figure S2**B**; mean±s.e.m; ΔF/F-integral is normalized to the average Δ F/F-integral of spontaneous spikes). As expected, the non-CF signal got smaller as the threshold decreased; however, we still observed a significant non-CF signal at all the thresholds (one sample t-test $P<0.05$). Figure S2**C** represents the average non-CF trace for threshold 1 (red) and 0.5 (blue), demonstrating that even when the rate of missed calcium spikes is very low, i.e. at threshold 0.5, a clear non-CF signal could be detected. All data in this figure came from GCaMP6f experiments. We observed similar results for OGB-1/AM data.