

Supplemental Material

Research Articles | Systems/Circuits

Tuft dendrite spikes are accompanied by selective input from distinct functional networks

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Supplemental Figure Legends

Figure S1. Activity timecourse processing, rates, and robustness. **A**, Intra-trial photobleaching for example session. Whole-image trace (black) and double exponential decay model fit (dashed green; $\tau_{fast} = 23.0\text{ ms}$, $\tau_{slow} = 287\text{ ms}$; $R^2 = 0.99$). **B**, Mean of trial-averaged time courses across all synapses (no pre-processing) with (blue) and without (green) bleach correction for session in (A) ($n = 64$ synapses). **C**, Event rates of tuft spikes and synaptic $\theta(\Delta F/F_0)$ events ($n = 313$ dendrites; $n = 2072$ synapses). **D**, Event amplitudes for tuft spikes ($n = 3312$ spikes). **E**, Mean Ca^{2+} event transient across dendrites using a low ($0.3\Delta F/F_0$; olive; $n = 313$) and high ($1\Delta F/F_0$; gray; $n = 194$) minimum event amplitude threshold. **F**, Same as (E) except mean synaptic activity aligned to calcium transients ($\theta(\Delta F/F_0)_{TSA}$; low $n = 826$; high $n = 333$). **G**, Mean pre-onset (-575–0 ms) and post-onset (0–575 ms) activity for data in (F). **H**, Mean inferred motion (black) and after largest motion frames were removed (dark red; $n = 52$ sessions). **I**, Mean synaptic $\theta(\Delta F/F_0)$ activity aligned to tuft spikes before (black) and after (dark red) removing largest motion frames ($n = 826$ synapses). **J**, Mean pre-onset (-575–0 ms) and post-onset (0–575 ms) activity for data in (I). **K**, Average mean-subtracted $\theta(\Delta F/F_0)_{TSA}$ activity aligned to putative multi-branch tuft spikes. SD synapses (cyan; $n = 485$). OD synapses (gray; $n = 1332$). **L**, Mean pre-onset (-575–0 ms) and post-onset (0–575 ms) activity for data shown in (K). Error shading and error bars are hierarchical bootstrap SEM. Statistical tests were two-tailed hierarchical difference tests (see Table 6).

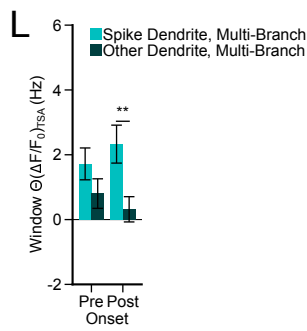
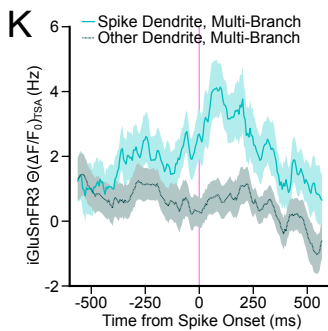
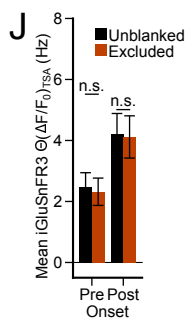
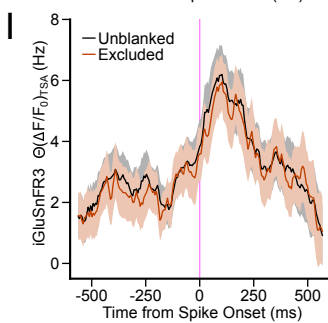
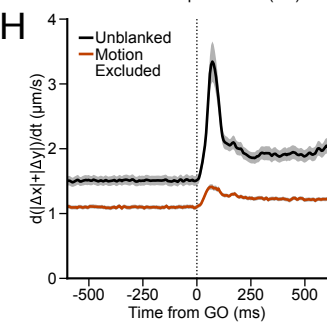
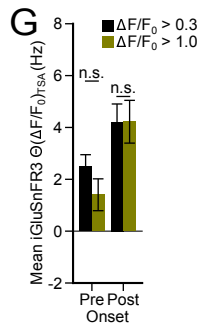
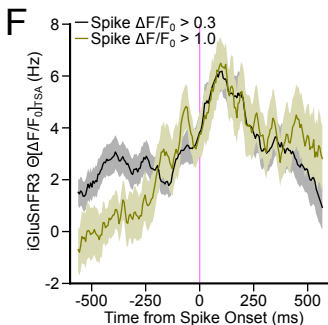
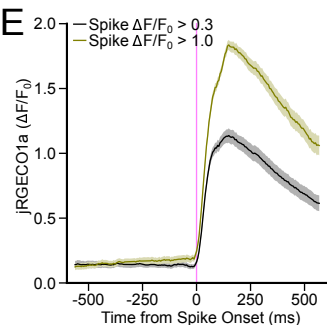
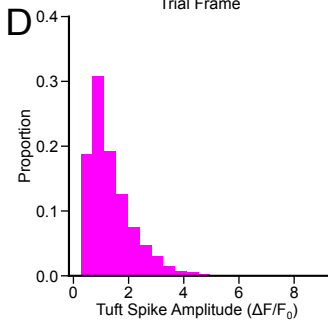
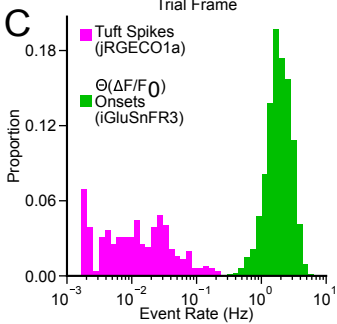
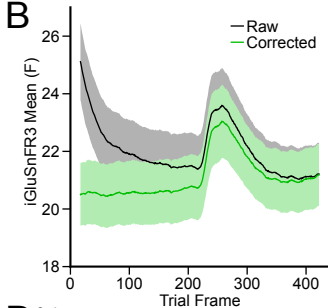
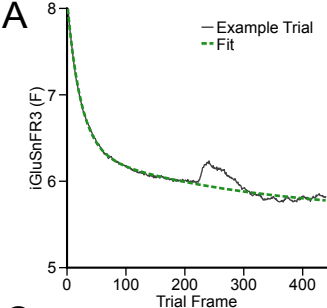
Figure S2. Functional synaptic clustering analysis. **A**, Mean pairwise synaptic noise correlations (ρ) as a function of branch or Euclidean distance ($\theta(dF/dt)_{TSA}$). Pairs located on the same dendrite (black; $n = 6674$ pairs; $R^2 = 0.99$) or on different dendrites (gray; $n = 47775$ pairs; $R^2 = 0.98$). **B**, Same as (A), except for signal correlation ($\theta(dF/dt)$) for within dendrite pairs (black; $R^2 = 0.96$) and across dendrite pairs (gray; $R^2 = 0.76$). **C**, Mean pairwise synaptic population coupling similarity for synapses located on the same or different dendrites ($n = 461$ synapses; two-tailed hierarchical bootstrap mean difference test $p = 0.19$). Error shading and error bars: hierarchical bootstrap SEM.

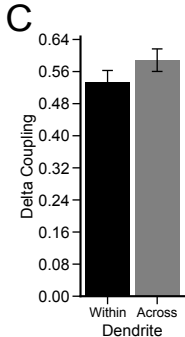
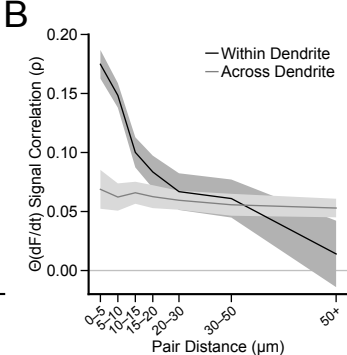
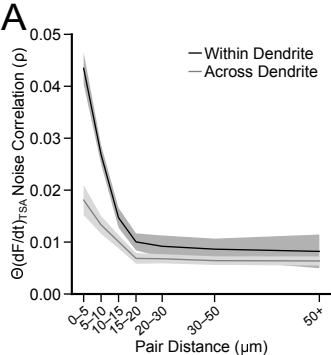
Figure S3. Robustness of population coupling estimates and organization. **A**, Relationship between coupling rank and population size used to calculate rank. ($r = 0.12$; $n = 2591$ synapses). **B**, Relationship between synaptic coupling ranks when combining all sessions together versus ranks calculated using single sessions. Red line indicates linear fit ($r = 0.83$; $n = 2591$ synapses). **C**, Relationship between coupling rank and average individual $\Delta F/F_0$ transient size ($r = -0.18$; $n = 2591$ synapses). **D**, Relationship between synaptic coupling ranks before and after removing the top 15% of inferred high-motion frames (see Methods for further details). Red line indicates linear fit ($r = 0.99$; $n = 2591$ synapses). **E**, Mean correlation of synapse pairs by population coupling similarity during spontaneous behavior using $\Delta F/F_0$ traces including pairs in the same coupling range (black circles; $n = 1965$ pairs) and different coupling ranges (gray triangles; $n = 22424$ pairs). Error bars: hierarchical

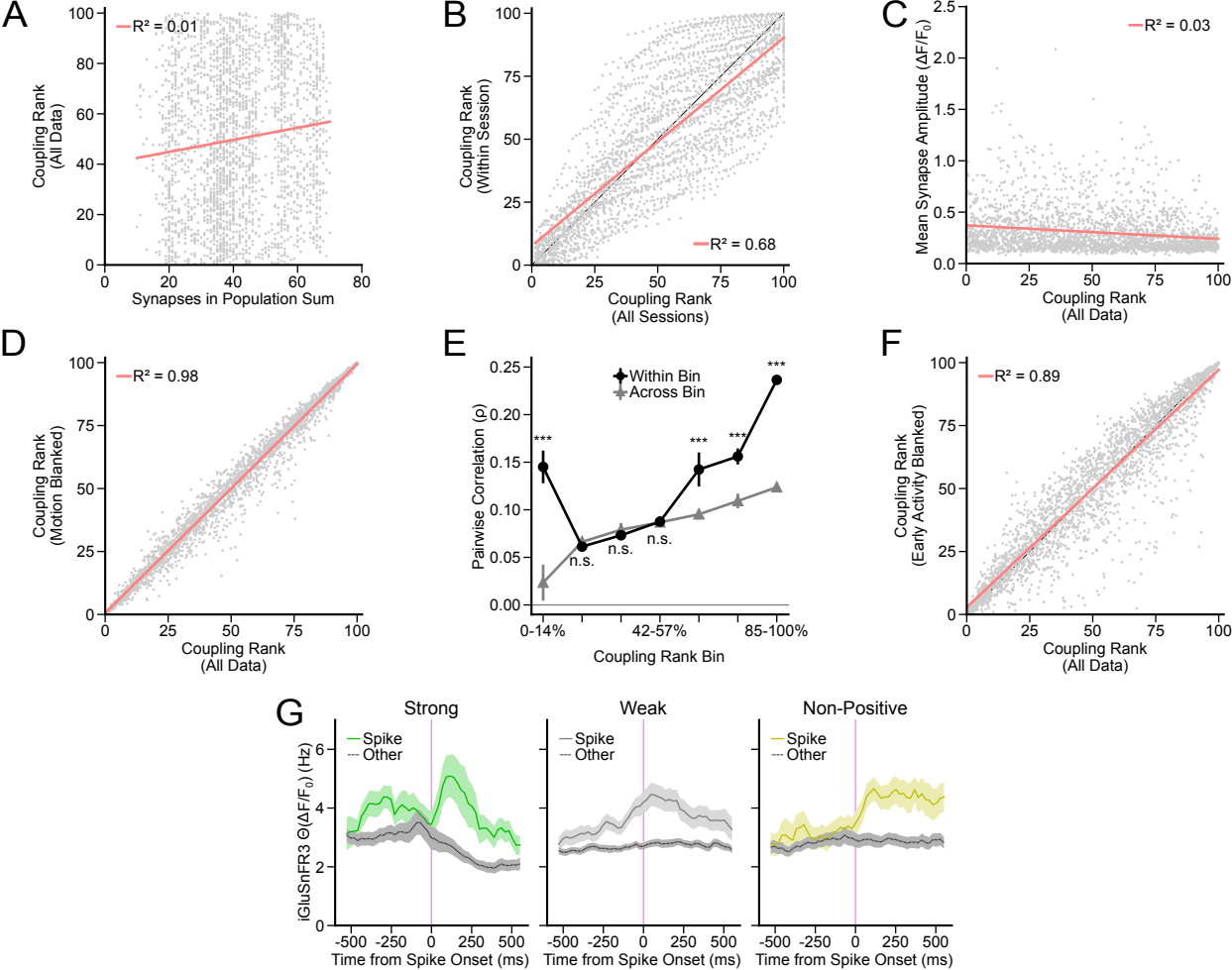
bootstrap SEM. Statistical significance: one-tailed hierarchical bootstrap mean difference test (see Table 7). **F**, Relationship between coupling rank before and after removal of the early response period (0–0.5 s from GO cue) from analysis. Red line indicates linear fit ($r = 0.94$; $n = 2591$ synapses). **G**, $\theta(\Delta F/F_0)$ synaptic SD activity (solid lines) and OD activity (dashed lines) aligned to tuft spikes for synapses grouped by coupling strength (n : $SC_{SD} = 144$; $SC_{OD} = 382$, $WC_{SD} = 522$; $WC_{OD} = 1156$; $NC_{SD} = 160$; $NC_{OD} = 384$).

Figure S4. Similar organization of population coupling in silicon probe recordings. **A**, Secondary data analysis measuring spiking activity at single neuron resolution in ALM using silicon probes in a similar behavioral task (Li et al., 2015). **B**, Spiking activity noise correlation (ρ) as a function of coupling similarity. Black circles represent pairs in the same coupling range ($n = 753$ pairs). Gray triangles represent pairs in different coupling ranges with one neuron belonging to a given range ($n = 6586$ pairs). Error bars: hierarchical bootstrap SEM. Statistical significance: one-tailed hierarchical bootstrap mean difference test (see Table 8).

Figure S5. Additional characterization of task coding and population coupling groups. **A**, Trial-averaged mean $\theta(\Delta F/F_0)$ aligned to GO cue (dotted black line) for correct (C), incorrect (I), correct right (CR), and correct left (CL) trial conditions for strong (SC), weak (WC) and nonpositive (NC) coupling groups (C/CR/CL n : $SC = 518$, $WC = 1555$, $NC = 518$ synapses; I n : $SC = 479$, $WC = 1267$, $NC = 434$ synapses). **B**, Visual depiction of activity mode calculation. **C**, Population mean along the GO mode, Response direction, and Outcome direction activity for synapses. $\theta(\Delta F/F_0)$ traces used for calculation. Gray shading indicates the epoch used to calculate activity-mode weights. GO Mode (-0.25–0.25 s) projected onto CR and CL trials. Response Direction (0–0.6 s) projected onto CR and CL trials. Outcome Direction (0.6–1.2 s) projected onto correct and error trials (GO Mode and Response Direction $n = 2591$ synapses; Outcome Direction $n = 2180$ synapses). **D**, Same as (C), except split up by population coupling group (GO Mode and Response Direction n : $SC = 518$, $WC = 1555$, $NC = 518$; Outcome Direction n : $SC = 479$, $WC = 1267$, $NC = 434$ synapses). Error shading: hierarchical bootstrap SEM.

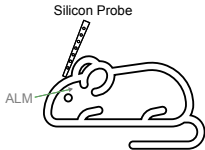






A

Li, Nuo, et al., 2015 *Nature*

**B**