SUPPLEMENTARY INFORMATION

Olfactory cortical neurons read out a relative time code in the olfactory bulb

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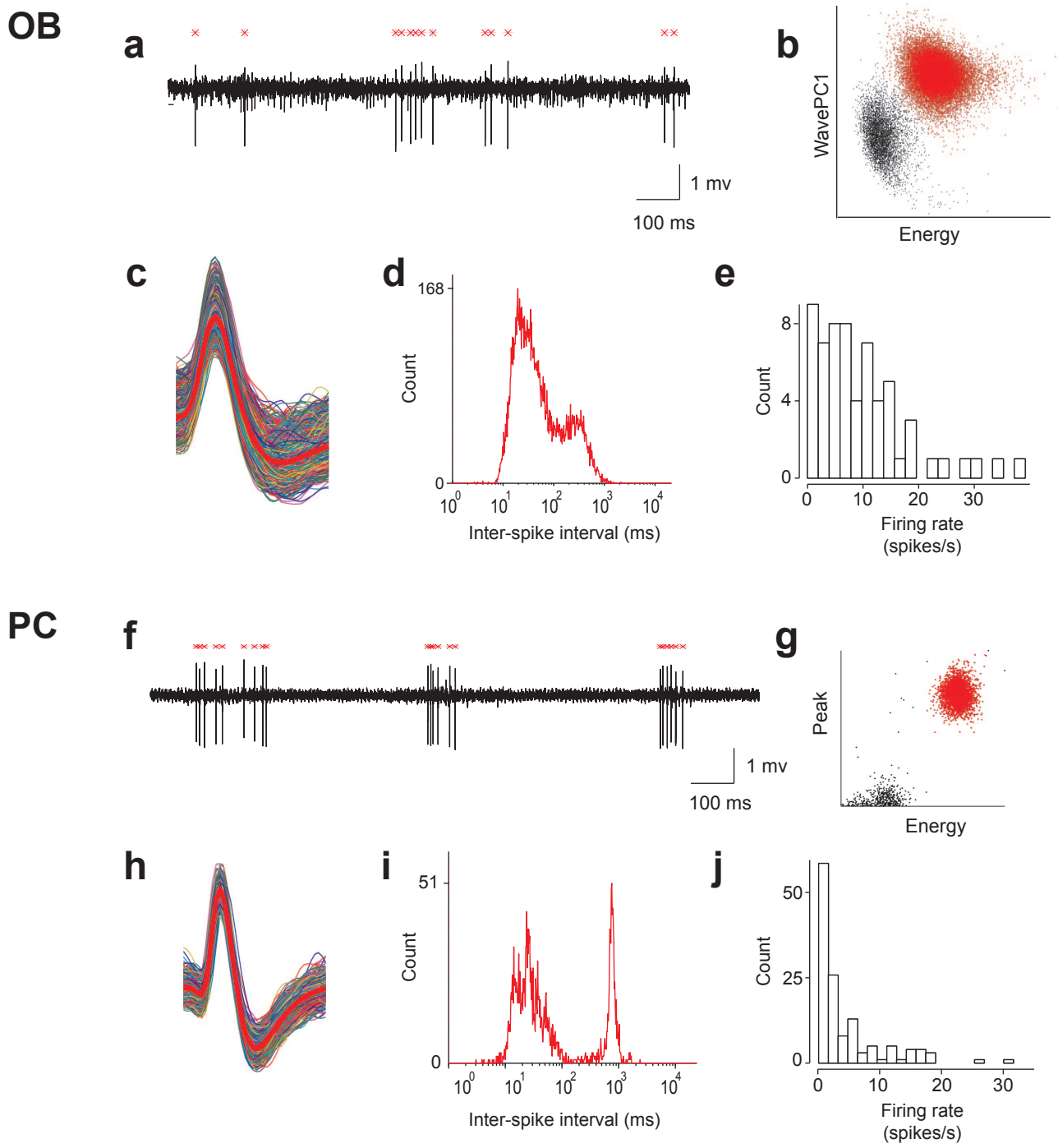
Supplementary Figure 1-10.



Supplementary Figure 1| Neural recording

(**a**) An example voltage trace of recording from an M/T cell. Red asterisks indicate identified spikes. OB, olfactory bulb.

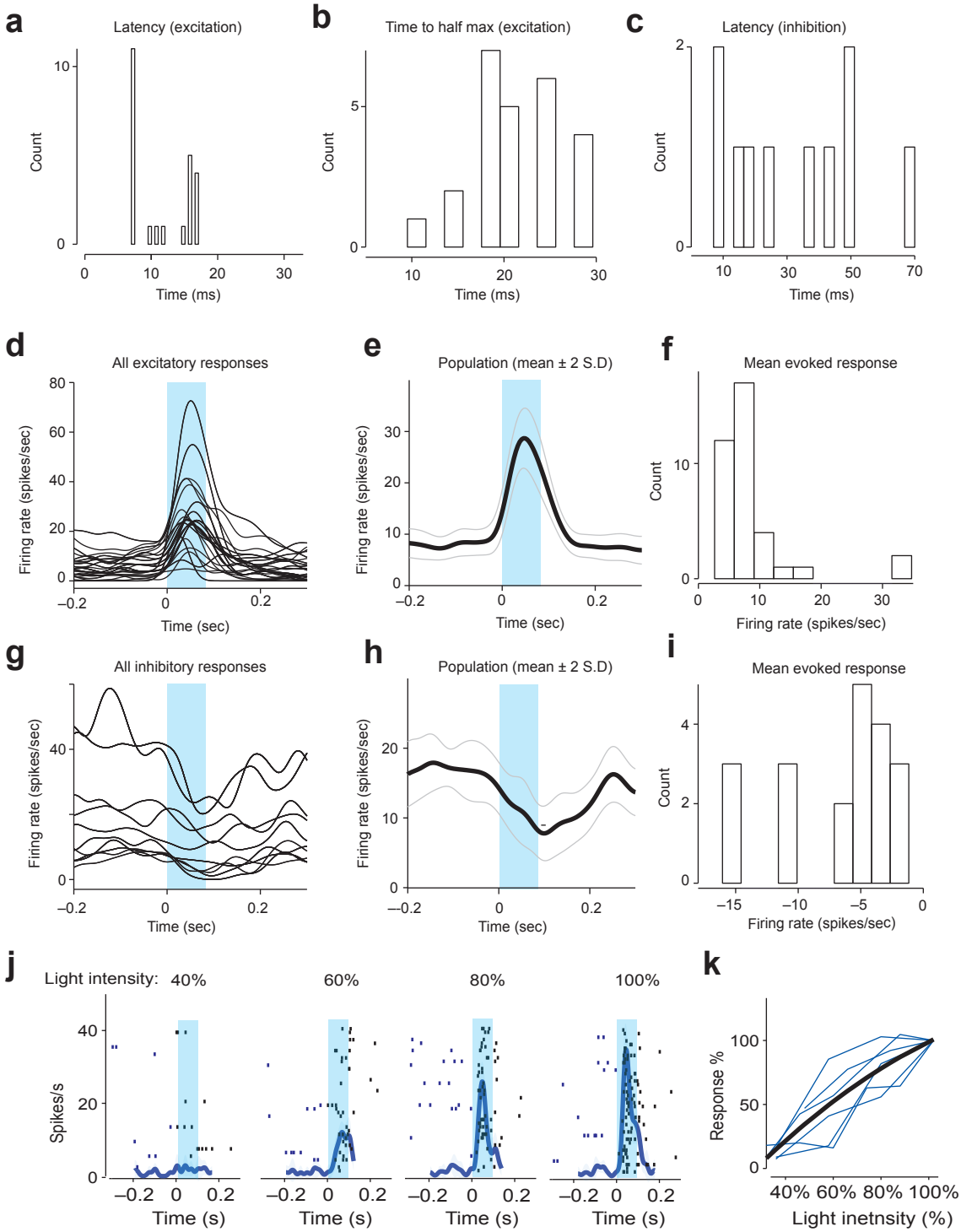
(**b**) Scatter plot for spike isolation. A plot using energy and the first principle component (wavePC1) of spike waveforms is shown. Red is an isolated single unit. Black is a noise cluster.

(**c**) Spike waveforms from all recorded spikes from the isolated single unit in **1a** and **1b**. The thick red line represents the mean waveform.

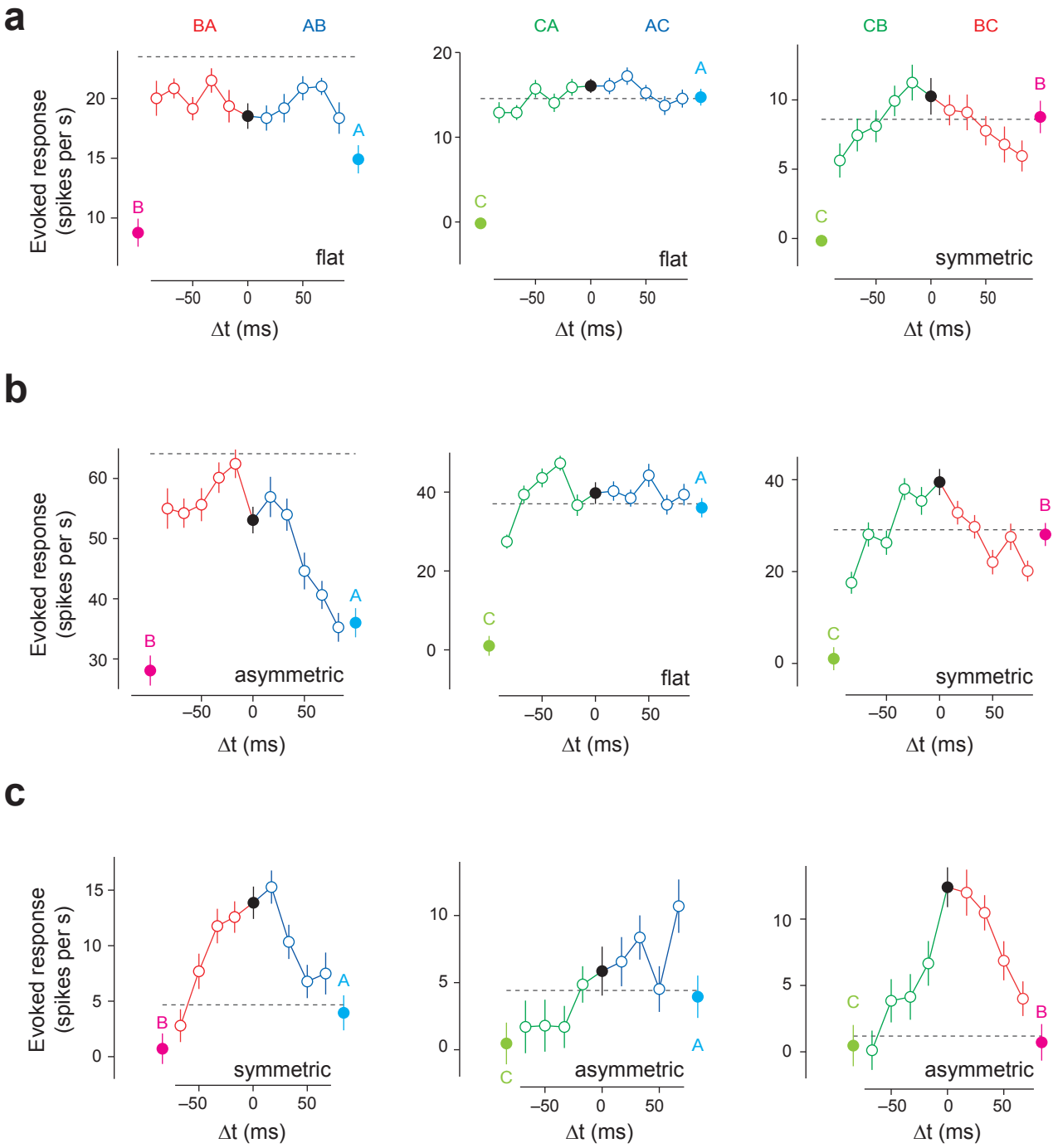
(**d**) Inter-spike interval distribution of the example neuron. Very few spikes occurred during the refractory period (inter-spike interval < 3ms). This neuron was well-isolated as measured by the L-ratio (8.0×10^{-6}) (Schmitzer-Torbert and Redish, 2004).

(**e**) Distribution of the average spontaneous firing rate for all OBNs in our data set.

(**f-j**) Data from PCNs. PC, piriform cortex. The example neuron was well-isolated as measured by the L-ratio (1.7×10^{-9}).



Supplementary Figure 2| Optogenetic activations of M/T cells in OMP-ChR2 mice
(**a**) Distribution of latency for all M/T cell-single spot combinations that showed statistically significant excitations ($P < 0.05$, *t*-test, n = 25 M/T cell-single spot combinations). Latency is defined as the time at which the number of spikes in a 20 ms window is significantly larger than the number of spikes in 200 ms before stimulation. (**b**) Time to reach the half maximum firing rate of the averaged PSTH. (**c**) Distribution of latency for all M/T cell-single spot combinations that showed statistically significant inhibitions ($P < 0.05$, *t*-test, $n = 10$ M/T cell-single spot combinations). (**d**) Temporal dynamics of all M/T cell excitatory responses. The timing of light stimulation is indicated by the cyan bar. (**e**) Average time course of excitatory responses. The gray lines represent ± 2 s.e.m.. (**f**) Distribution of evoked excitatory responses calculated in a 200 ms window from light onset.(**g**) Temporal dynamics of all M/T cell inhibitory responses. (**h**) Average time course of inhibitory responses. The gray lines represent ± 2 s.e.m.. (**i**) Distribution of evoked inhibitory responses calculated in a 200 ms window from light onset. (**j**) Optical activations of OB neurons with varying intensity. Raster plots (black ticks) and PSTHs (blue lines) of an example M/T cell using varying light intensity. (**k**) Normalized responses of 6 M/T cells to varying light intensity (blue). Black line: the polynomial fit to all data points.

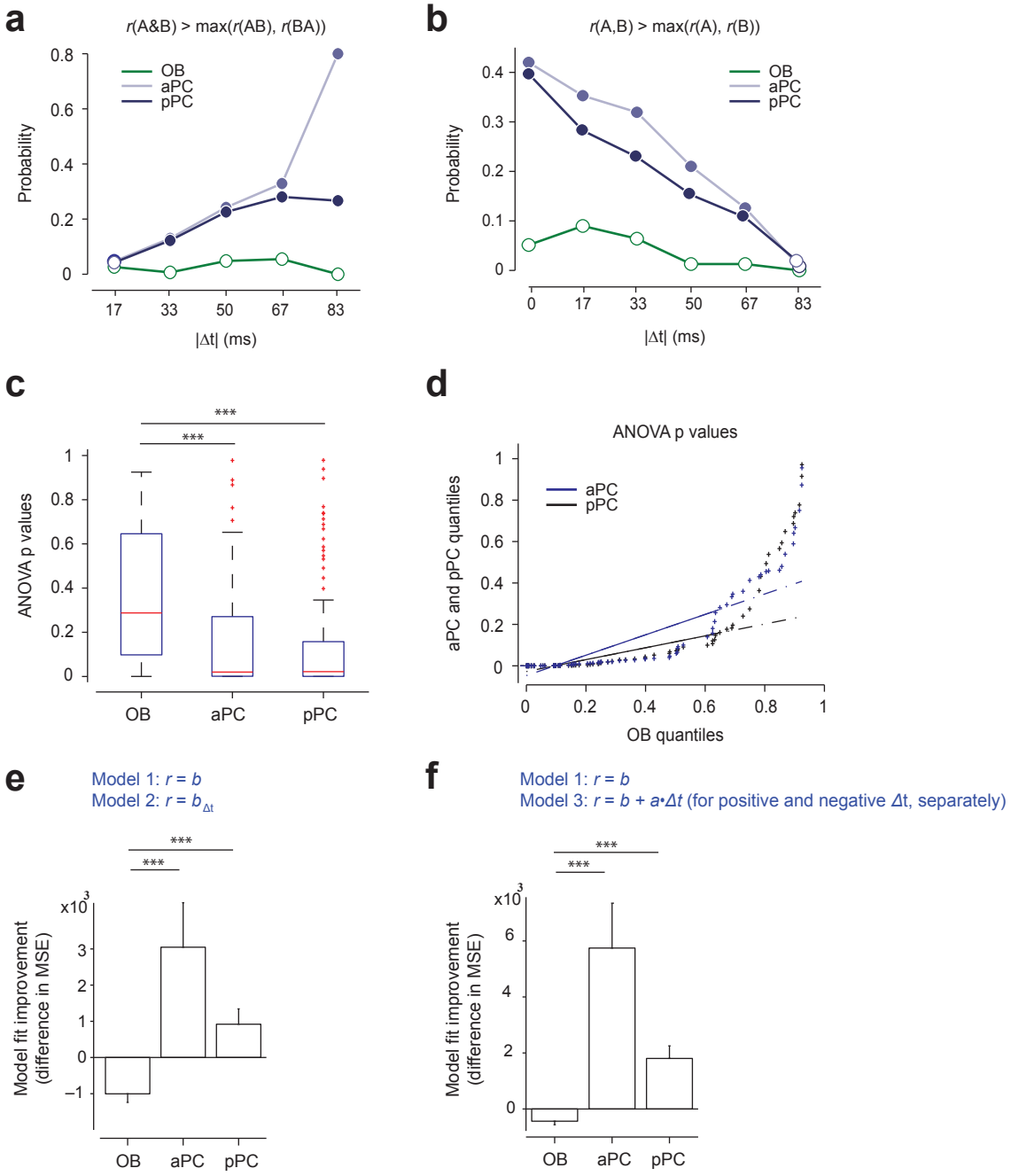


Supplementary Figure 3| TTCs from multiple spot pairs in single neurons

(**a**) TTC from an example pPC neuron. This neuron was examined using three pairs of spots in the olfactory bulb (A/B, A/C and B/C). Spots A and B interacted sublinearly (left panel, the responses to A→B and B→A, were less than the sum of the responses to spot A and B for all lags – dashed line). Spot A and C did not show apparent interactions (middle panel, the response is similar to the response to spot A for all lags). Spots C and B produced a TTC with a symmetric, inverted-V shape (right panel). Spot C affected the neuron when combined with spot B but not when combined with spot A.

(**b**) TTC from an example pPC neuron. Spot A and B produced an asymmetric TTC (left panel, the response to A→B is decreasing with increasing lag but the same for B→A). Spot A and C resulted in a symmetric TTC (middle panel, the response is similar to the response to spot A for all lags). Spot B and C resulted in a TTC with symmetric, inverted V-shape (right panel). Spot C affected this neurons' response only when combined with spot B but not with spot A.

(**c**) TTC from an example pPC neuron. Spot A and B caused a TTC with a symmetric, inverted V-shape (left panel). Spot A and C produced an asymmetric TTC with a lag-specific facilitatory interaction (middle panel, the response to A→B is higher than B→A at Δt = 67, $t_{78} = 2.7$, $P = 0.0088$). Spot B and C produced a TTC that is globally asymmetric (right panel, $F_{1,316} = 4.2$, $P_A = 0.042$, ANCOVA, $n = 40$ repetitions). All three combinations of spots affected the recorded neuron.



Supplementary Figure 4| Relationships between simultaneous, lagged and single spot stimulations

(**a**) Probability of TTCs in which the response to simultaneous stimulation (r(A&B)) was stronger than that to lagged stimulation of the two spots (r(AB) or r(BA)). The results are plotted separately for each lag.

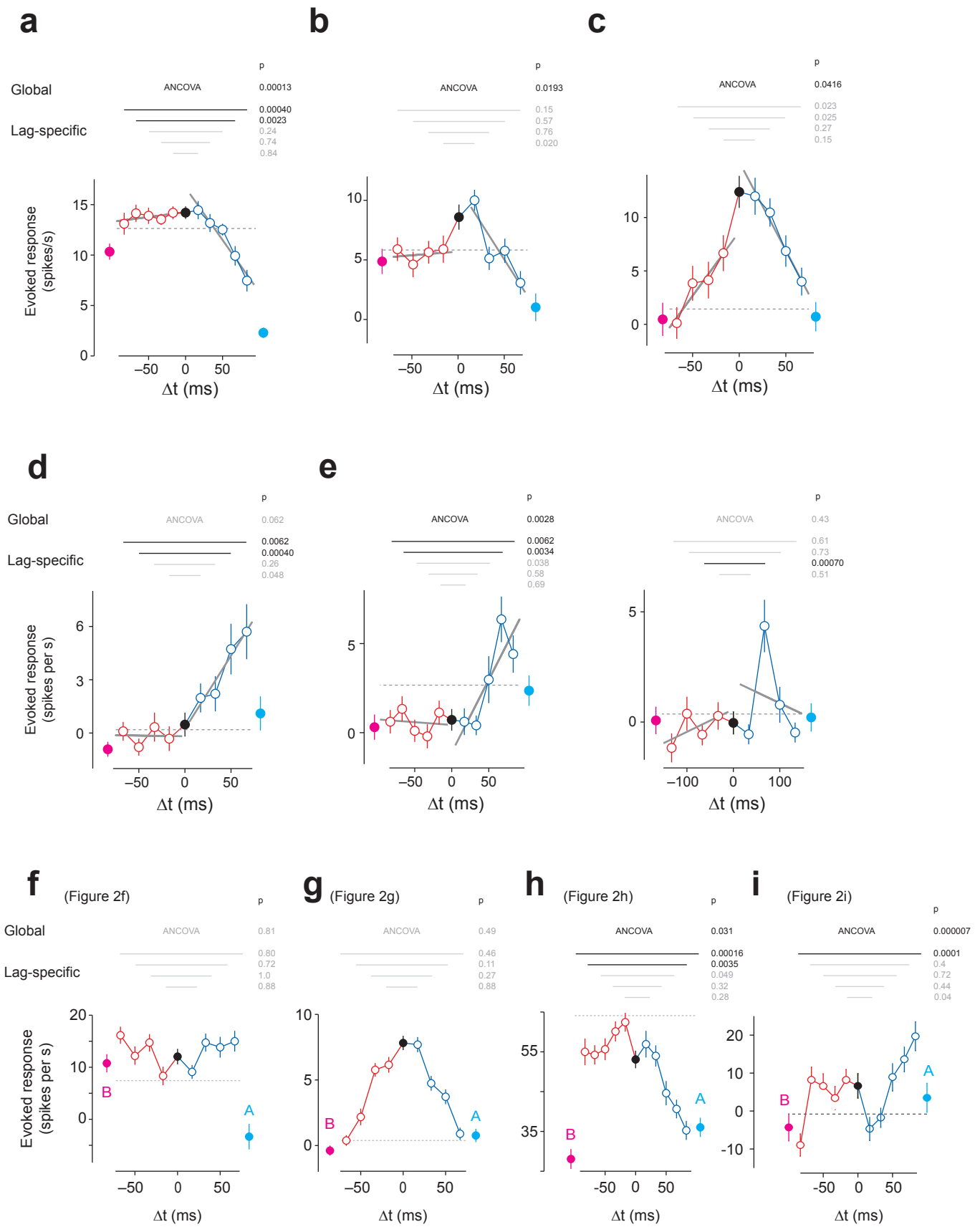
(**b**) Probability of TTCs in which the response to lagged stimulation of two spots (r(A,B)) was stronger than that of both of the single spot stimulations (r(A) or r(B)).

(**c**) Distribution of p-values for ANOVA with time lags as a main factor. For each TTC, we performed ANOVA with time lags as a main factor, and obtained a p-value. If responses were modulated by Δt (i.e. not flat), then p-values should be small. The p-value distribution for the olfactory bulb neurons was significantly higher than those for aPC or pPC neurons ($P < 0.001$, t-test).

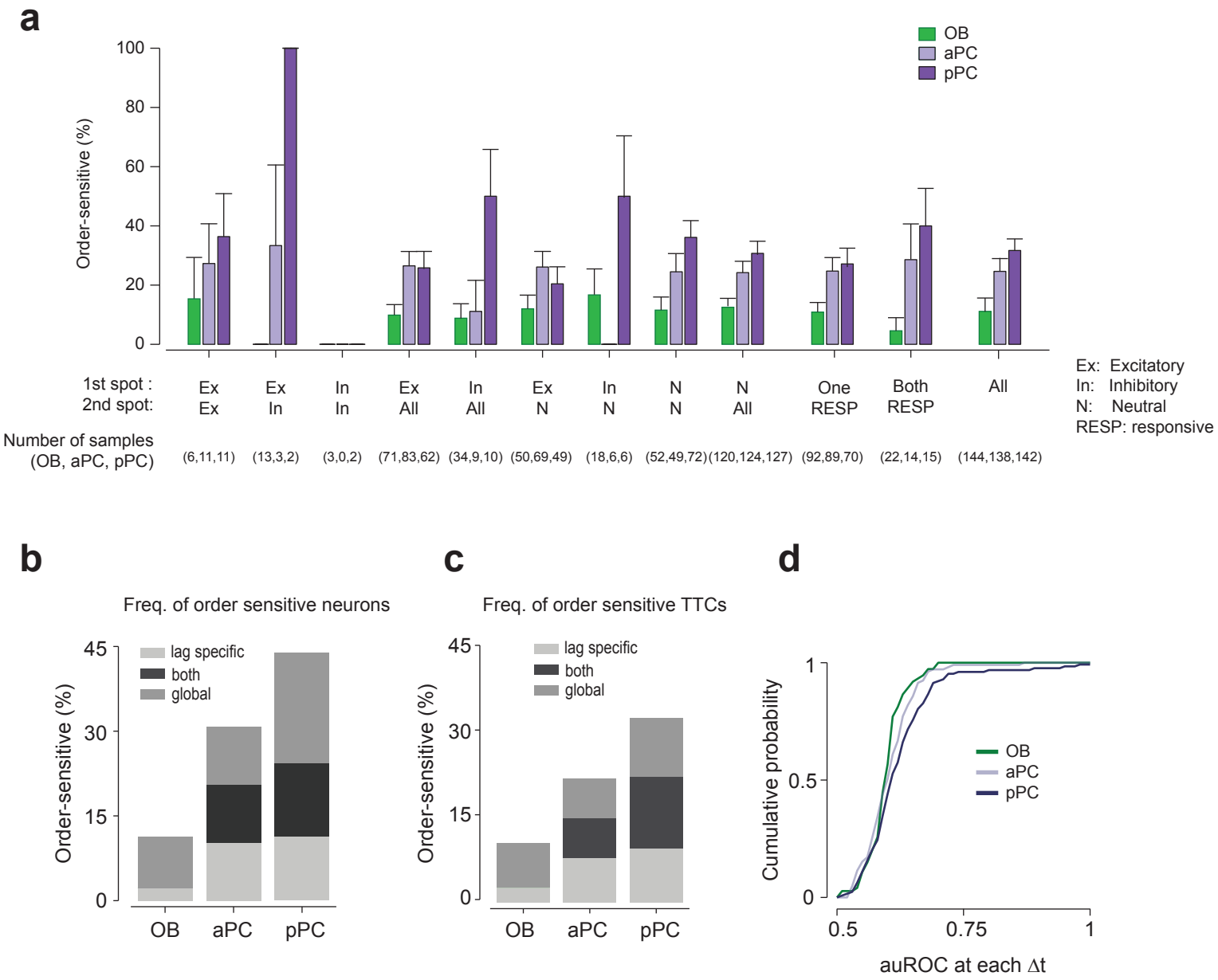
(**d**) A quantile plot of the p-values in (**c**). Superimposed on the plot is a line joining the first and third quartiles of each distribution.

(**e**) The improvement of model fit using a model that takes into account Δt. The two models compared are shown on the top. The model 1 takes into account only the global mean of the responses (b) whereas the model 2 takes into account the mean for each Δt. If neural responses are flat with respect to Δt, we should expect smaller improvement using the model 2 compared to the model 1. Consistent with the main result, PC neurons showed larger improvements by the model 2. ***, $P < 0.001$, Mann-Whitney U test.

(**f**) The improvement of model fit using a model that takes into account Δt. The two models compared are shown on the top. The model 3 uses a linear fit separately for positive and negative Δt. If neural responses are flat with respect to Δt, we should expect smaller improvement using the model 3 compared to the model 1. Consistent with the main result, PC neurons showed larger improvements by the model 3. ***, $P < 0.001$, Mann-Whitney U test.



Supplementary Figure 5| Statistical analysis of example TTCs
The results of statistical tests on the TTCs shown in **Figs. 4** and **2**. The p-values for global asymmetry ($P_A < 0.05$, ANCOVA) are displayed at the top of each panel. The p-values for lag-specific asymmetry are indicated by the horizontal lines drawn above the TTCs. Significant differences are indicated by black while non-significant cases are indicated by gray ($P < 0.05$ divided by the number of lags tested, Bonferroni correction).



Supplementary Figure 6| Frequencies of order sensitive TTCs

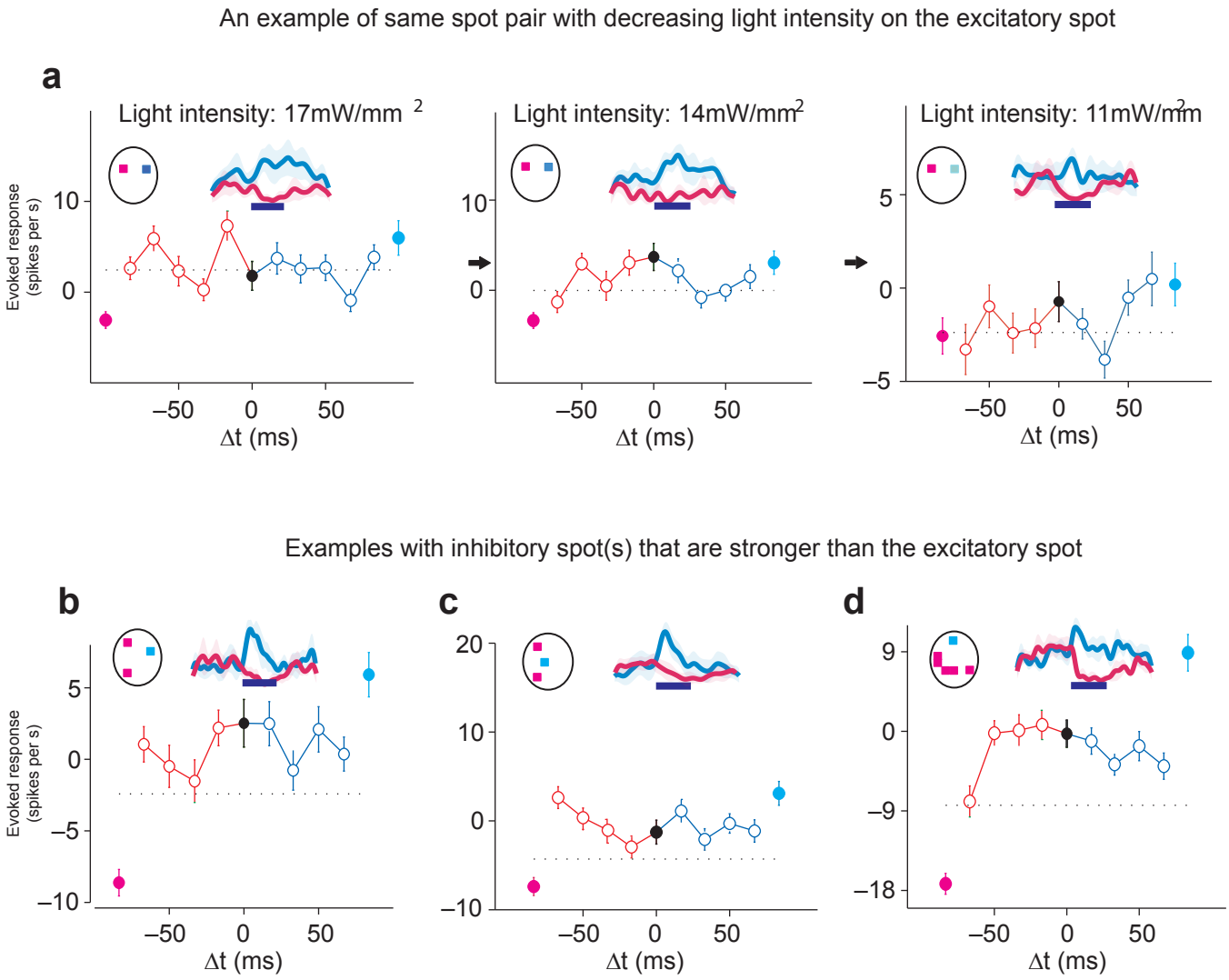
(**a**) Frequencies of order sensitive TTCs as a function of spot-pair types. Each spot was categorized into excitatory (Ex), inhibitory (In) or neutral (N) based on single spot stimulations ($P < 0.05$, *t*-test). We also show the results when one spot was responsive (either excitatory or inhibitory; RESP) and when both were responsive. The number of TTCs in each category and brain region is indicated below. Experiments using OMP-ChR2 and Tbet-cre mice were pooled.

(**b**) Percentage of neurons with lag-specific and globally asymmetric TTCs.

(**b**) Percentage of lag-specific and globally asymmetric TTCs.

(**d**) Cumulative distributions of the magnitudes of lag-specific differences in evoked responses.

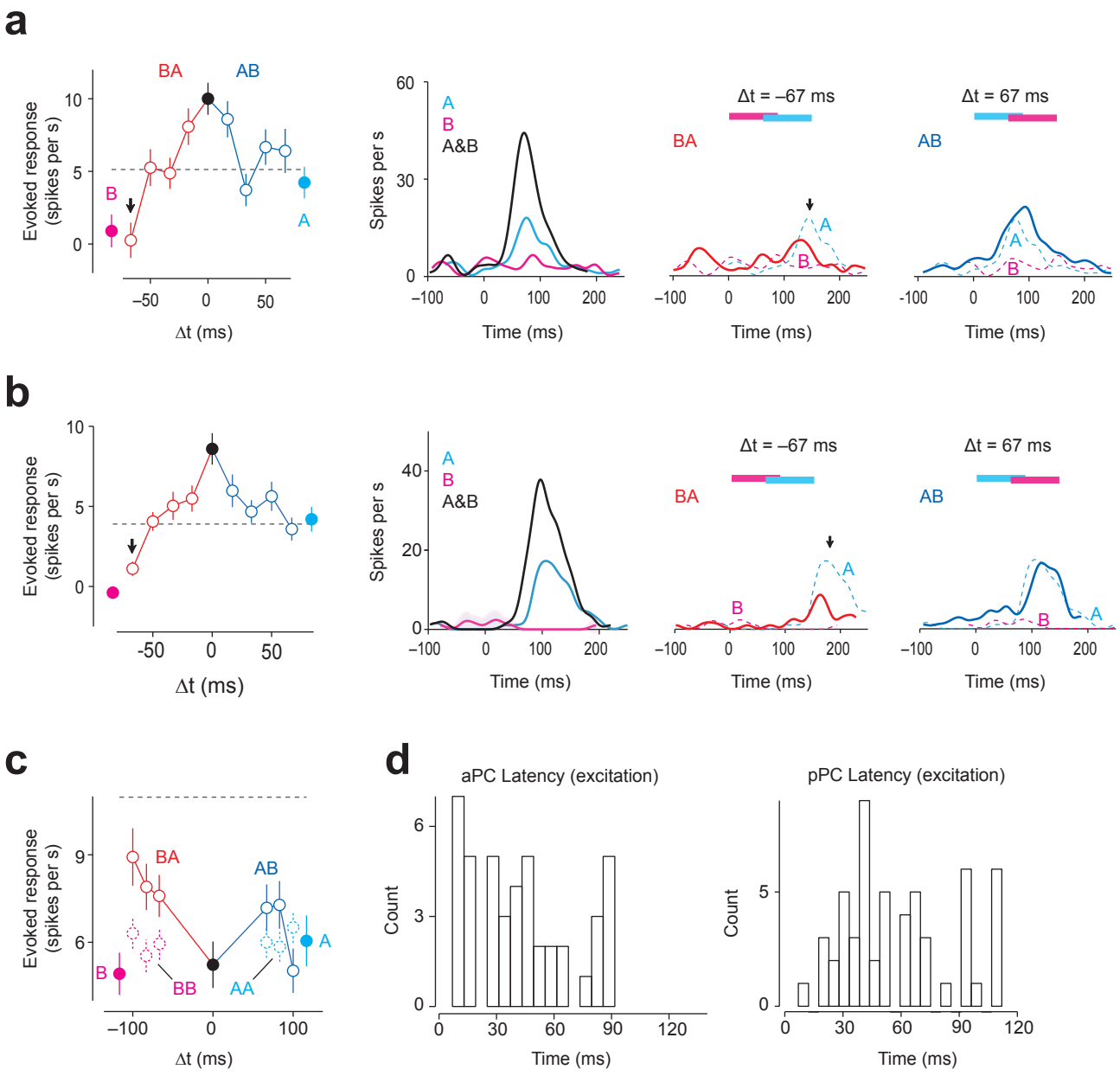
For a pair of positive and negative lags, the area under the receiver operating characteristics curve (auROC) was obtained for the two distributions of evoked response. The data includes 301, 420 and 527 pairs of Δt's obtained in 76,114, 129 spot pairs in OB, aPC and pPC, respectively.



Supplementary Figure 7| Control experiments using reduced light intensity

(**a**) 3 TTCs of one M/T neuron generated using the same spot pair. Light intensity on the excitatory spot was reduced from 17mW/mm^2 to 11mW/mm^2. All TTCs were symmetric (tests for both lag-specific and global asymmetry). Inset: Excitatory (cyan) and inhibitory (red) PSTHs and spot locations.

(**b-d**) 3 TTCs from 3 different neurons using different spot pairs. Multiple inhibitory spots were selected to generate strong inhibition. The resulting TTCs were not asymmetric. Inset: Excitatory (cyan) and inhibitory (red) PSTHs and spot locations.



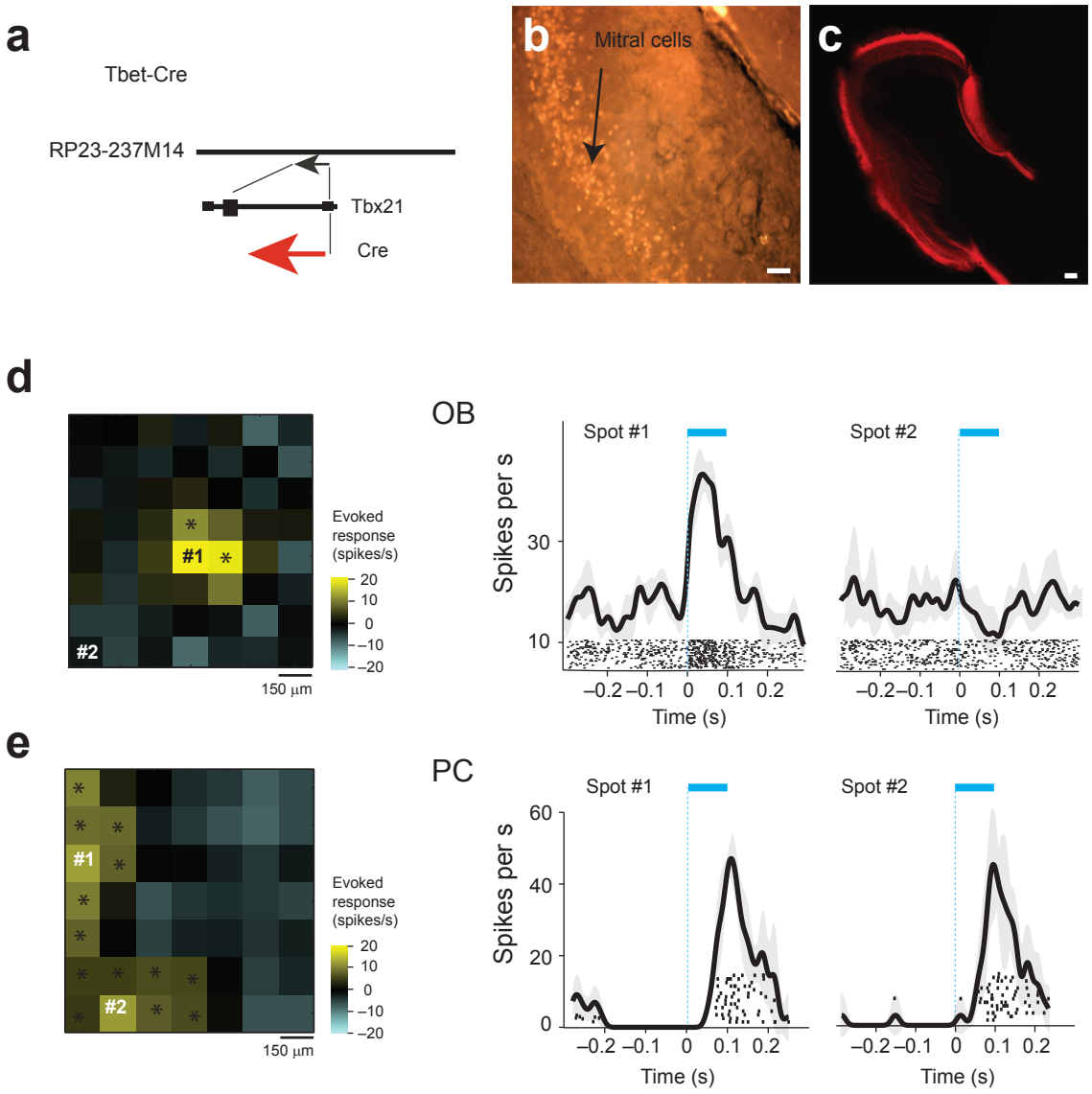
Supplementary Figure 8| Delayed inhibition of response shapes the responsivity of piriform cortex neurons

(**a**) TTC of an example pPC neuron (first panel). This neuron was excited by spot A but not by spot B. Second panel, PSTHs of the stimulations with spots A, B and A&B. The response of this neuron decreased steeply with increasing Δt for B→A but less so for A→B. Global asymmetry, $F_{1,316} = 4.2$, $P_A = 0.042$ (ANCOVA); lag-specific asymmetry, $t_{78} = 3.1$, $P = 0.0023$ (*t*-test for Δt = 67 ms, $n = 40$ repetitions). The third and fourth panels show the responses for Δt = −67 and 67 ms, respectively. Dashed lines are the expected responses to the stimulation of the corresponding spot. The second stimulation was effective in evoking responses with Δt = 67 ms (right panel), but not with Δt = -67 ms (third panel, black arrow).

(**b**) A similar example as in (a) of a pPC neuron. This neuron was excited by spot A and inhibited by spot B. Global asymmetry, $F_{1,316} = 3.0$, $P_A = 0.084$ (ANCOVA); lag-specific asymmetry, $t_{78} = 2.6$, $P = 0.0099$ (*t*-test for Δt = 67).

(**c**) TTC of a pPC cell that peaked at a non-zero lag. Both spots A and B are excitatory (magenta and cyan). The response to B → A with Δt = 100 ms is significantly higher than the response for A&B ($t_{78} = 2.8$, $P = 0.005$, *t*-test). The dashed blue and red circles show the responses to repeated stimulations of either A or B (AA or BB) with a given lag (Δt). This experiment shows that the facilitation observed for B→A at Δt = 100 ms is not due to facilitation resulting from the repeated activation of the recorded neuron but specific to the combination (and the order) of A and B (B→A).

(**d**) Distribution of latency for all excitatory aPC and pPC neurons. Only spot-neuron combinations that showed significant responses were used ($P < 0.05$, *t*-test). aPC, 42 32 ms; pPC, 50 27 ms (mean S.D., $n = 36$ and 41 spot-neuron combinations, in aPC and pPC respectively).



Supplementary Figure 9| Generation and characterization of Tbet-cre mice

(**a**) Schematic of the construct for Tbet-cre BAC transgenic mice. The final, recombined BAC was confirmed to be free of any gross rearrangements by restriction analysis, linearized, and injected into B6/CBA oocytes. Of fourteen resulting pups, one genotyped was positive for the Tbet-cre transgene. This founder line was confirmed to strongly express CRE by anti-CRE antibody staining, and to induce recombination within the mitral and external tufted cell layers of the main and accessory olfactory bulbs. A survey of the rest of the brain shows that Tbet-cre induced recombination is specific to the olfactory bulbs. No other sites of expression were observed in the brain. Outside of the brain, we observed Tbet-cre induced recombination in the thymus, a known site of Tbet/Tbx21 expression (Faedo et al., 2002). The kinetics of Tbet-cre expression and ability to induce recombination in the olfactory bulb are consistent with the known kinetics of endogenous Tbet expression, already present at the first differentiation of mitral cell layer from the budding olfactory bulb in the E14.5 embryonic brain (Faedo et al., 2002).

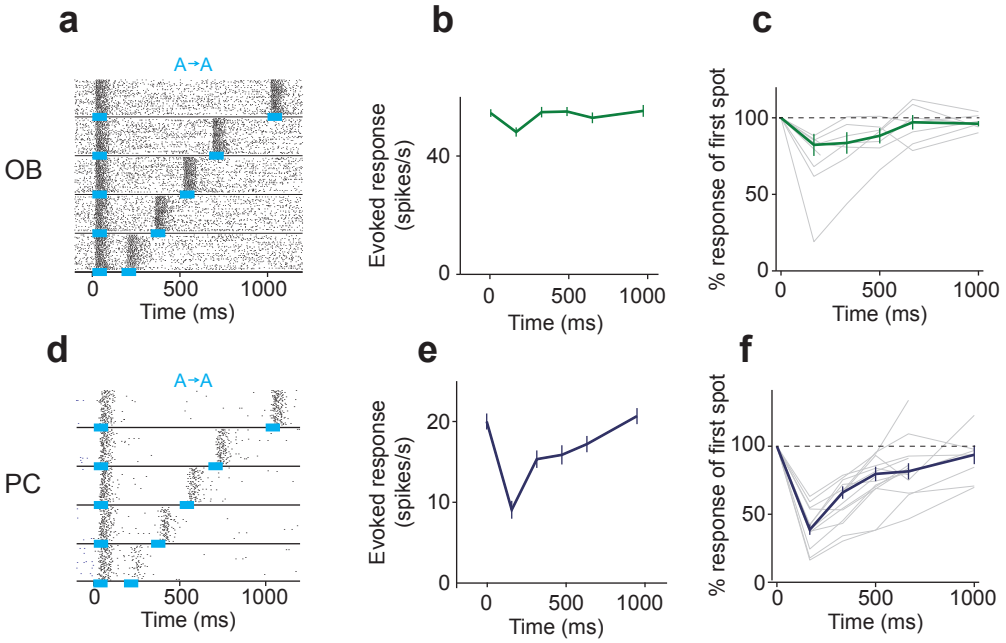
(**b**) Cre expression in the mitral cell layer of the accessory olfactory bulb in an adult mouse by anti-Cre antibody staining.

(**c**) Distribution of ChR2-tdTomato in a sagittal section across a mouse brain.

(**d**) Left, Two-dimensional light activation map for an example neuron in olfactory bulb (OB). Conventions are as in **Fig. 1**. Values are the average of 20 randomly interleaved repetitions. This neuron was excited by 3 spots and ($P < 0.05$, *t*-test, Bonferroni correction, asterisks).

(**e**) Similar to (**d**) for PCN. OB was optically stimulated while recording from a PCN. PCNs tended to respond from several spots. There was no clear inhibitory response in this example recording. Values are the average of 15 randomly interleaved repetitions. This neuron was excited by 15 spots ($P < 0.05$, *t*-test, Bonferroni correction, asterisks).

Faedo, A. et al. Developmental expression of the T-box transcription factor T-bet/Tbx21 during mouse embryogenesis. ***Mech Dev*** 116, 157-60 (2002).



Supplementary Figure 10| OB Ns and PCNs response to repeated light stimulation
(**a-c**) Experiments in which a single spot (duration: 83 ms) was stimulated twice with varying lags while recording from M/T cells. Raster plot (**a**) and average responses (**b**) for a single M/T cell and for a population of M/T cells (**c**, $n = 8$ neurons). Average firing rate was obtained using a 200 ms window from light onset. The cyan bars in d indicate the timing of light stimulation. Black horizontal lines separate between different Δt's. Gray lines in f are from each experiment. Green lines, Mean s.e.m.
(**d-f**) Experiments in which a single spot was stimulated twice with varying lags while recording from PC cells. Raster plot (**d**) and average responses (**e**) for a single PCN, and a population of PCNs(**f**, $n = 14$ neurons). Average firing rate was obtained using a 200 ms window from light onset. Gray lines in i are from each experiment. Purple lines, Mean s.e.m..