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Supplemental Data

In Vivo Light-Induced Activation of Neural Circuitry in Transgenic Mice Expressing Channelrhodopsin-2

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Figure S1. Neither Granule nor Periglomerular Cells of the Olfactory Bulb Express ChR2-YFP

(A-C) Thin sections (10 μm) taken through glomeruli highlighting the absence of ChR2-YFP expression in surrounding periglomerular cells as detected by lack of colocalization with Pax6 (long arrows). ChR2-YFP expression is limited to mitral cell dendrites coursing through the section (green, short arrows). The white dashed line indicates a glomerulus.
(D-F) Thin sections through the glomerular layer highlighting the absence of ChR2-YFP expression in surrounding periglomerular cells as detected by lack of colocalization with calbindin (Calb, red, arrows). ChR2-YFP expression is limited to mitral cell dendrites (green). The white dashed lines indicate the glomeruli. Scale bar: 20 μm.
(G-I) High magnification view of a glomerulus showing the apical dendrite of a mitral cell (long arrow) in association with nearby periglomerular cells (PG, short arrows). The periglomerular cells are labeled with GAD67 (red) and do not show colocalization with ChR2-YFP (green). Note the high levels of membrane-associated ChR2-YFP in the mitral cell apical dendrite.
(J-L) High magnification view of granule cell bodies residing in the internal plexiform layer (IPL) marked with an antibody against GAD67 (red) showing a lack of ChR2-YFP expression (long arrows). ChR2-YFP expression can be detected in centrifugal fibers (f, short arrows) of the IPL. Scale bars: 20 μm (A-C); 20 μm (D-F); 10 μm (G-I); 10 μm (J-L).

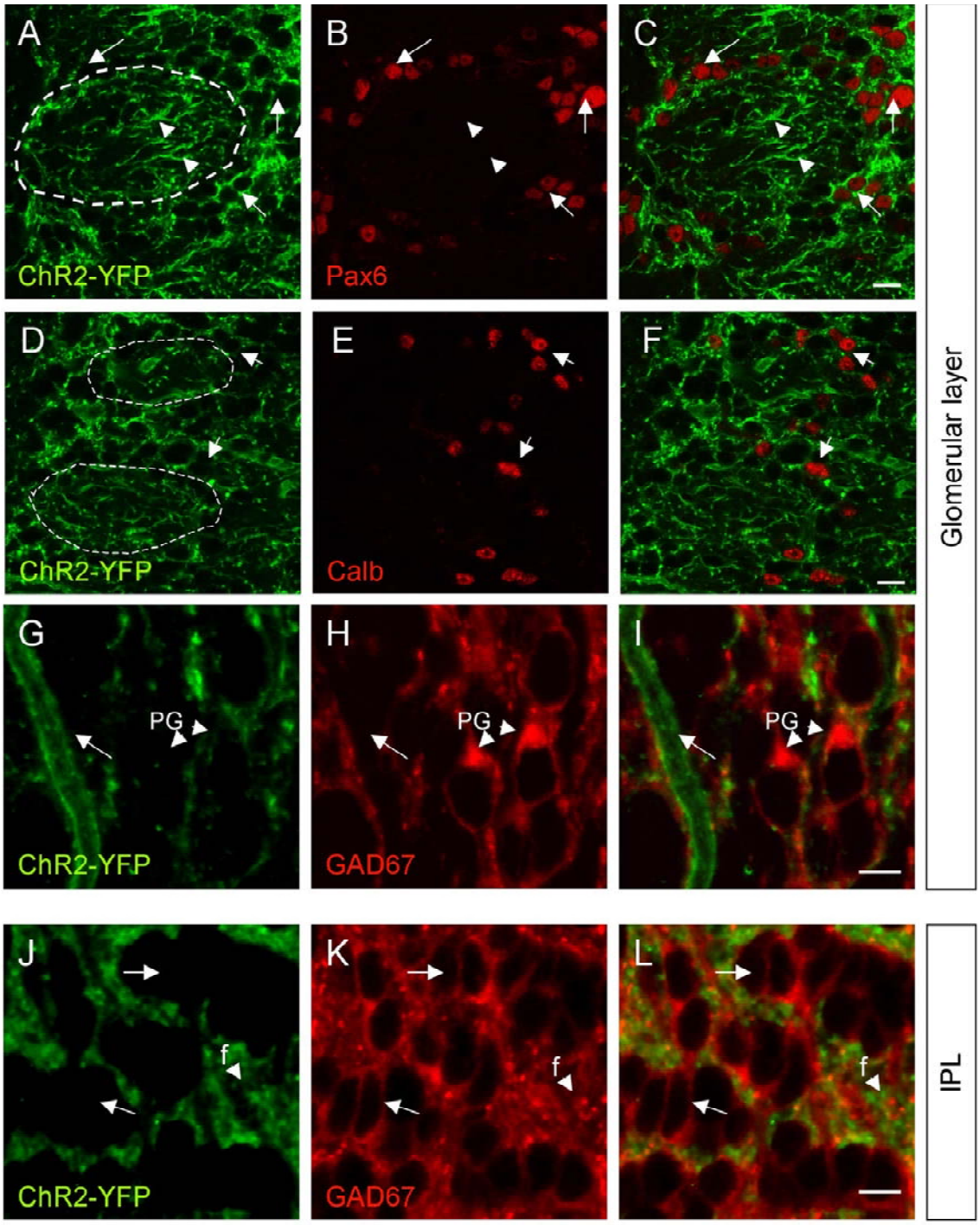
A
B
C
ChR2-YFP
Pax6
D
E
F
ChR2-YFP
Calb
Glomerular layer
G
H
I
PG
PG
PG
ChR2-YFP
GAD67
J
K
L
f
f
f
ChR2-YFP
GAD67
IPL

Figure S2. Light-Responsive Mitral Cells are Stimulated by Odors

(A) Physiological recordings monitoring heart and breathing rates during mitral cell stimulation.
(B) Output channels marking the sequential light (blue) activation and odor (gold) stimulation with the concentrated volatile odorant isoamyl acetate.
(C) Electrophysiological activity recorded from mitral cell units upon sequential light and odor stimulations as indicated in (B). Top (gray) traces correspond to sorted spike events from the raw electrophysiological trace shown at the bottom (black). Note that exposure to isoamyl acetate (odor) elicited a sustained increase in respiration-locked firing of the isolated light-responsive mitral cell.

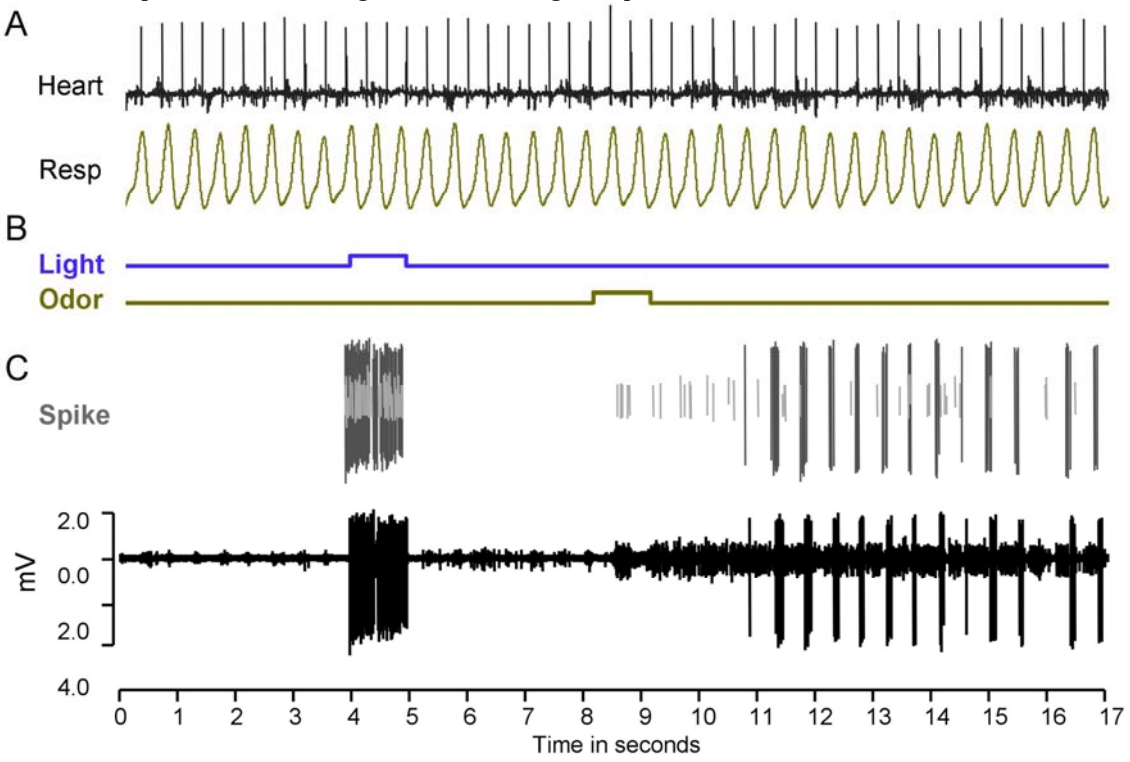

Figure S3. Remote Focal Illumination of the Bulb Surface Does Not Influence the Firing Rates of Individual Mitral Cells

(A) (Top) Diagram of the olfactory bulb showing an example recording site and the corresponding spots of either direct (left), or indirect (right) illumination. (Bottom) Example traces showing the light-evoked increase in mitral cell firing using a spot of light 100 µm in diameter focused directly over the recorded cell (left), or the lack of light-evoked change in mitral cell firing with indirect illumination (right). The numbered spots on the surface of the bulb shown on the right depict the different positions that were illuminated away from the recorded cell using a spot of light 300 µm in diameter. Scale bar: 1 mm. (B-F) Quantitative comparisons of mitral cell responses to direct illumination and to indirect illumination away from the recording site. Data represent values from 9 different cells. (B) Mitral cell firing rates increase with direct illumination using a spot of illumination 100 µm in diameter. (C) Mitral cell firing rates increase upon presentation of the volatile concentrated odorant isoamyl acetate. (D) No change in mitral cell baseline firing rate with indirect illumination using a spot of light 300 µm in diameter at multiple positions away from the recording site. (E) Odor-evoked mitral cell firing rate does not change with indirect illumination using a spot of light 300 µm in diameter at multiple positions away from the recording site. (F) Direct light-evoked mitral cell firing rate does not change with simultaneous indirect illumination using a second spot of light 300 µm in diameter at multiple positions away from the recording site.