**Cell Reports, Volume *32***

## Supplemental Information

# Visual Input into the *Drosophila melanogaster* Mushroom Body

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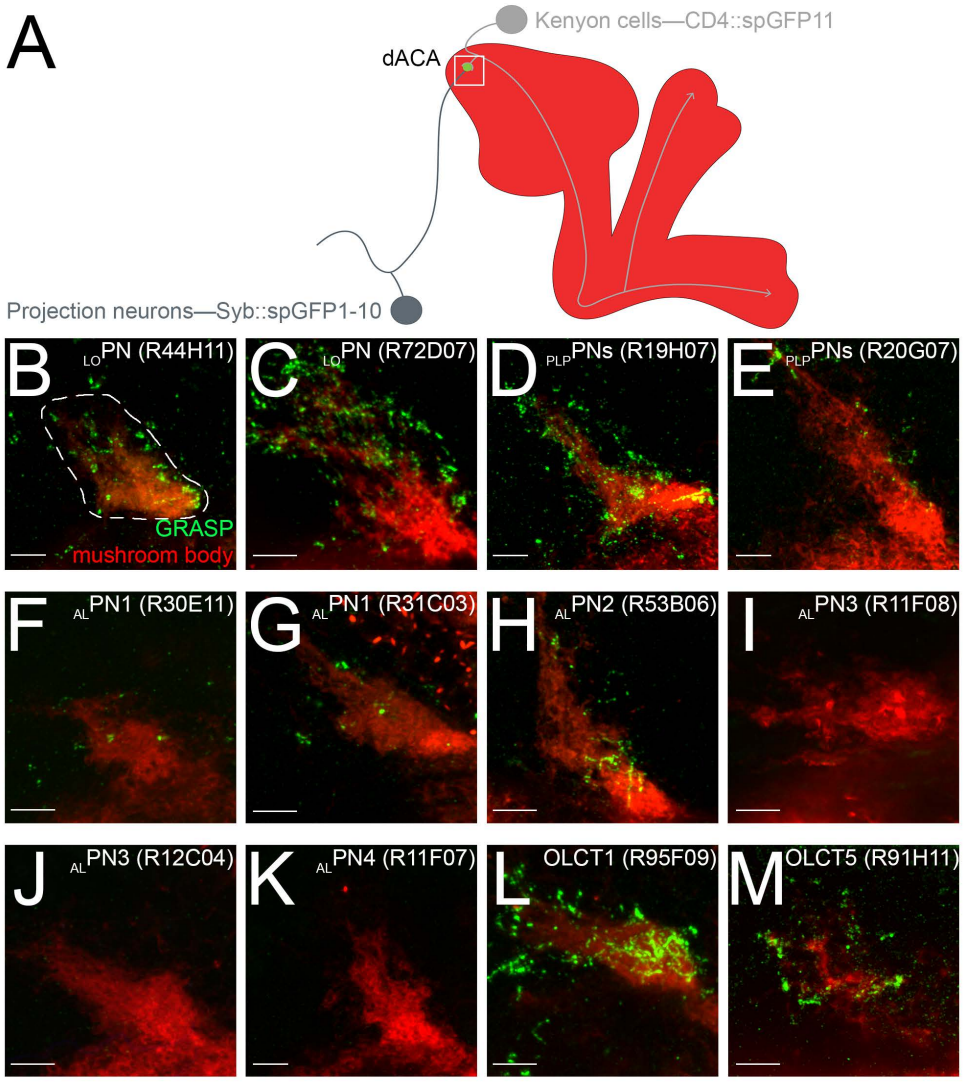
A
Kenyon cells—CD4::spGFP11
dACA
Projection neurons—Syb::spGFP1-10
B
LO PN (R44H11)
GRASP
mushroom body
C
LO PN (R72D07)
D
PLP PNs (R19H07)
E
PLP PNs (R20G07)
F
AL PN1 (R30E11)
G
AL PN1 (R31C03)
H
AL PN2 (R53B06)
I
AL PN3 (R11F08)
J
AL PN3 (R12C04)
K
AL PN4 (R11F07)
L
OLCT1 (R95F09)
M
OLCT5 (R91H11)

**Figure S1. Identification of the transgenic lines driving expression in neurons presynaptic to the $\alpha/\beta_p$ Kenyon cells. Related to Figure 3.** (A) A schematic of the *Drosophila* brain shows how the GFP Reconstitution Across Synaptic Partners (GRASP) technique was used to determine whether the selected transgenic lines drive expression in neurons that are presynaptic to the $\alpha/\beta_p$ Kenyon cells. The expression of the spGFP1-10 fragment was driven in the putative input neurons (dark grey) — using the various transgenic lines identified in the screen — and the expression of the spGFP11 fragment was driven in the mushroom body (red) using a transgenic line driving in most Kenyon cells; $\alpha/\beta_p$ Kenyon cell (light grey) can be distinguished from other Kenyon cells because they are the only Kenyon cells projecting to the dorsal accessory calyx. Reconstituted GFP molecules (here depicted by a green circle) are visible in the dorsal accessory calyx of the mushroom body (red) when the spGFP$^{1-10}$ fragment is expressed in synaptic partners. The dorsal accessory calyx was imaged (white square). (B-M) A strong (B-E, L-M) or weak (F-H) GRASP signal was detected in some of the selected transgenic lines; no GRASP signal was detected in other lines (I-K). The following genotypes were used in this figure: *yw*/*yw*;*UAS-syb::spGFP1-10*$^{unknown}$, *LEXAop-CD4::spGFP11*$^{unknown}$/*LEXAop-tdTomato*$^{attP5}$;*R_line-GAL4*$^{attP2}$(as indicated on the panel)/*MB247-LEXA*$^{unkown}$;. Scale bars are 5 μm in all panels.

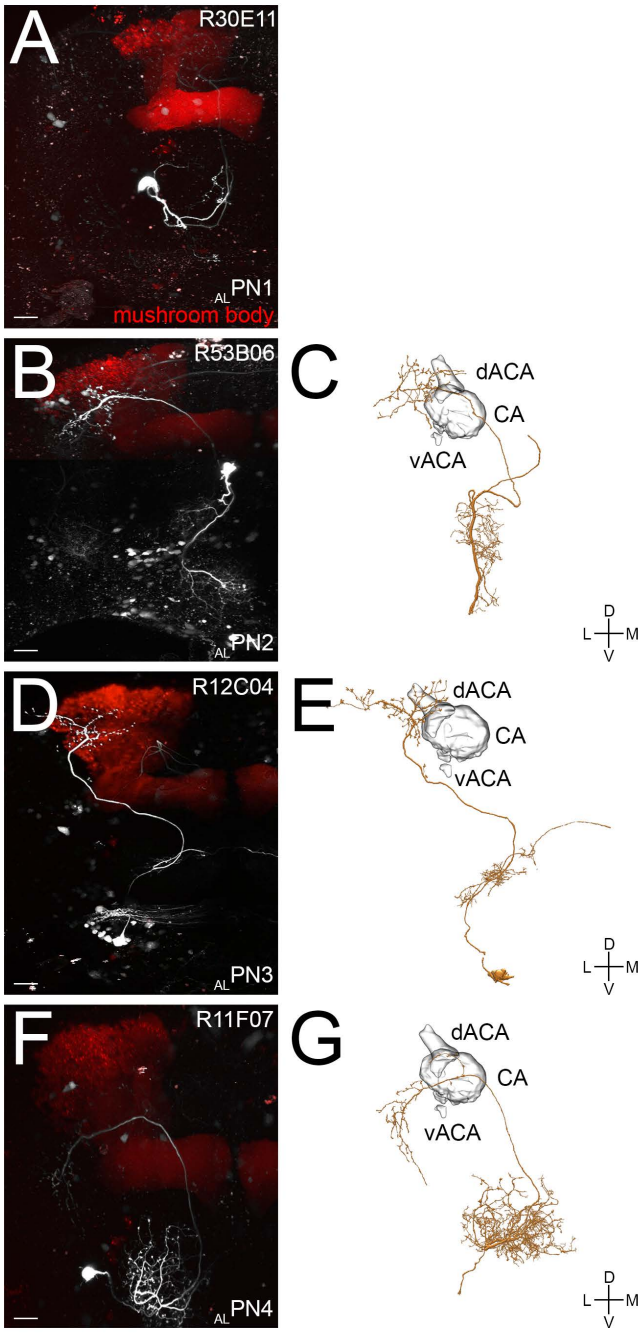
A
R30E11
AL
PN1
mushroom body
B
R53B06
AL
PN2
C
dACA
CA
vACA
D
L
M
V
D
R12C04
AL
PN3
E
dACA
CA
vACA
D
L
M
V
F
R11F07
AL
PN4
G
dACA
CA
vACA
D
L
M
V

**Figure S2. $_{AL}$PN projecting from the antennal lobe to region near the dorsal accessory calyx. Related to Figure 3, Figure S1.** (A, B, D, F) Four different neurons projecting from the antennal lobe to a region near the dorsal accessory calyx were identified using different transgenic lines (A: *R30E11-GAL4*, B: *R53B06-GAL4*, C: *R12C04-GAL4* and D: *R11F071-GAL4*); all the photo-labeled neurons project from the antennal lobe but each neuron has a distinct morphology. (C, E, G) Three of the four $_{AL}$PN-like neurons were identified in the hemibrain connectome. (A) $_{AL}$PN1 extends its dendrites in the posterior antennal lobe and projects its axons in the superior lateral protocerebrum. (B-C) $_{AL}$PN2 extends its dendrites into the column region of the posterior antennal lobe, a region known to be activated by high humidity, as well as in the sub-esophageal ganglion, a gustatory processing center, and projects its axons in the superior lateral protocerebrum. (D-E) $_{AL}$PN3 extends its dendrites into the arm region of the posterior antennal lobe, a region known to be activated by low humidity and projects its axons in the superior lateral protocerebrum. (F-G) $_{AL}$PN4 extends its dendrites broadly in the anterior antennal lobe, an olfactory processing center, and projects its axons in the posterior lateral protocerebrum. The images in (C), (E) and (G) were taken directly from the Neuprint platform. The neuropil domains are defined by Neurprint platform. The following genotypes were used in this figure: *yw*/*yw*;*MB247-DsRed*$^{unknown}$,*UAS-C3PA-GFP*$^{unknown}$/*UAS-C3PA-GFP*$^{attP40}$;*UAS-C3PA-GFP*$^{attP2}$,*UAS-C3PA-GFP*$^{VK00005}$,*UAS-C3PA-GFP*$^{VK00027}$/*R_line-GAL4*$^{attP2}$(as indicated on the panel);. Scale bars are 20 μm.

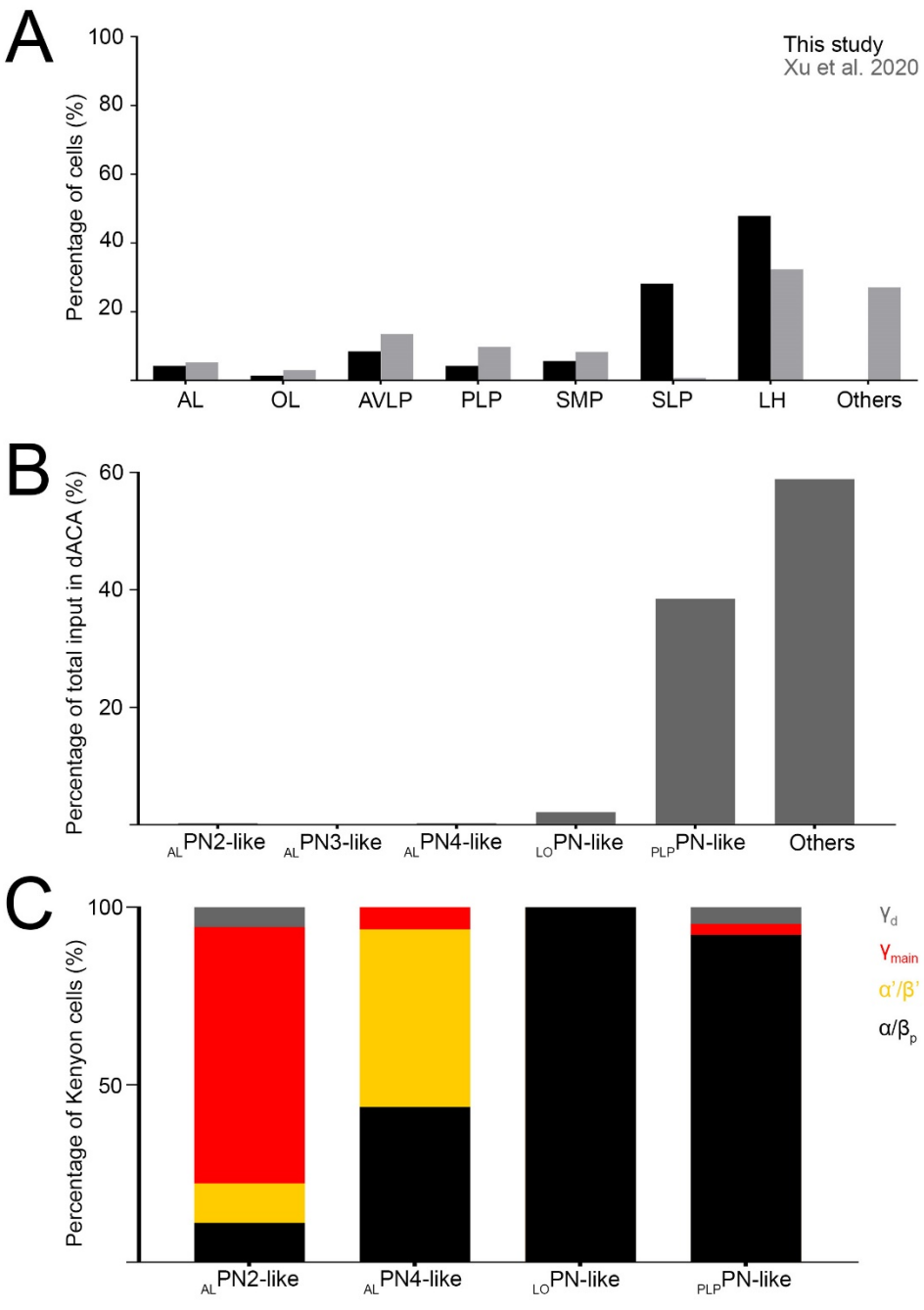
A
This study
Xu et al. 2020
Percentage of cells (%)
100
80
60
40
20
AL
OL
AVLP
PLP
SMP
SLP
LH
Others
B
Percentage of total input in dACA (%)
60
40
20
AL PN2-like
AL PN3-like
AL PN4-like
LO PN-like
PLP PN-like
Others
C
Percentage of Kenyon cells (%)
100
50
AL PN2-like
AL PN4-like
LO PN-like
PLP PN-like
γd
γmain
α'/β'
α/βp

**Figure S3. Identification of the projection neurons connecting to the $\alpha/\beta_p$ Kenyon cells in the dorsal accessory calyx in the connectome. Related to Figure 2, Figure 5.** (A-C) The 60 $\alpha/\beta_p$ Kenyon cells reconstructed in the *Drosophila* hemibrain connectome receive input from 133 neurons in the dorsal accessory calyx. (A) These input neurons can be divided into seven clusters based on the location of their cell bodies: the antennal lobe (AL), optic lobe (OL), anterior ventral lateral protocerebrum (AVLP), lateral horn (LH), superior lateral protocerebrum (SLP), superior medial protocerebrum (SMP) and posterior lateral protocerebrum (PLP) clusters; neurons with somata located outside these clusters are defined as "others". The percentage of neurons found in each cluster (grey) is similar to the one measured using the *en masse* photo-labeling technique reported in this study (black). (B) Among these 133 input neurons, neurons morphologically similar to $_{AL}$PNs, $_{LO}$PN and $_{PLP}$PNs were identified; the percentage of input $\alpha/\beta_p$ Kenyon cells receive in the dorsal accessory calyx from these different types of neuron varies across types. (C) $_{AL}$PN-like, $_{LO}$PN-like and $_{PLP}$PN-like neurons connect to different types of Kenyon cell; whereas $_{AL}$PNs connect mostly to olfactory Kenyon cells (namely $\gamma_{main}$ (red) and $\alpha'/\beta'$ (yellow) Kenyon cells), $_{LO}$PN-like and $_{PLP}$PN-like neurons connect mostly to $\alpha/\beta_p$ Kenyon cells (black). Very few connections to $\gamma_d$ Kenyon cells (grey) are found.

| Type of neuron | Location of cell body | Number of neurons | Driver line(s) | Connections to $\alpha/\beta_p$ Kenyon cells | |
|---|---|---|---|---|---|
| | | | | GRASP | Dye/photo-labeling technique |
| $_{LO}$**PN*** **(OCLT1)** | PLP cluster | 1 | R44H11 | strong signal | 4.1% (*n* = 27) |
| | | | R72D07 | strong signal | 3.0% (*n* = 27) |
| | | | R95F09 | strong signal | n/A |
| $_{PLP}$**PN** | LH cluster | 13 | R19H07 | strong signal | All: 14.1% (*n* = 24) Single: 1.9% (*n* = 32) |
| | | | R20G07 | weak signal | |
| $_{AL}$**PN1** | AL cluster | 1 | R30E11 | weak signal | 0 (*n* = 26) |
| | | | R31C03 | weak signal | |
| $_{AL}$**PN2** | AL cluster | 1 | R53B06 | weak signal | 0 (*n* = 30) |
| $_{AL}$**PN3** | AL cluster | 1 | R11F08 | no signal | 0 (*n* = 26) |
| | | | R12C04 | no signal | |
| $_{AL}$**PN4** | AL cluster | 1 | R11F07 | no signal | 0 (*n* = 25) |

**Table S1. Neurons projecting to the dorsal accessory calyx. Related to Figure 5, Figure S1.** The neurons identified in the screen were divided into three types — $_{LO}$PN, $_{PLP}$PN and $_{AL}$PN — based on the location of their dendrites — lobula (LO), posterior lateral protocerebrum (PLP) and antennal lobe (AL). The $_{AL}$PN type was further divided into four subtypes — $_{AL}$PN1, $_{AL}$PN2, $_{AL}$PN3 and $_{AL}$PN4 — based on the fact that each one of these neurons were identified using different transgenic lines. The asterisk demarks the only neuron identified by our study that is similar to a neuron described in the Yagi et al. (2016) study.

| Type of neuron (this study) | Type of neuron (Xu et al., 2020) | Neuprint ID number | Number of $\alpha/\beta_p$ Kenyon cells connected |
|---|---|---|---|
| $_{LO}$PN | PVL02b_pct (1) | 419908058 | 9 (2.14%) |
| $_{PLP}$PN (13) | PDL20y_pct (5) | 479969106 | 20 (4.99%) |
| | | 5813019543 | 10 (2.17%) |
| | | 450597298 | 6 (1.88%) |
| | | 389536926 | 13 (4.73%) |
| | | 5813009790 | 2 (0.32%) |
| | PDL23g_pct (4) | 5813011738 | 19 (6.91%) |
| | | 480310599 | 3 (0.26%) |
| | | 454335501 | 12 (4.12%) |
| | | 295461570 | 2 (0.42%) |
| | PDL20ob_pct (2) | 5813011717 | 8 (2.63%) |
| | | 511634742 | 3 (0.23%) |
| | PDL20z_pct (2) | 512023157 | 22 (6.61%) |
| | | 480997558 | 12 (3.21%) |
| $_{AL}$PN2 | VP multi adPN mALT | 5813063239 | 2 (0.26%) |
| $_{AL}$PN4 | olfactory multi lvPN mALT | 1037293275 | 4 (0.26%) |
| N/A | PVL10a_pct | 479935033 | 43 (15.14%) |

**Table S2. Input neurons of the dorsal accessory calyx as identified in the *Drosophila melanogaster* hemibrain connectome. Related to Figure 6, Figure S3.** The neurons connecting to $\alpha/\beta_p$ Kenyon cells in the dorsal accessory calyx — and their corresponding neurons, as described in this study — are listed; the numbers in parentheses indicate the number of neurons reported for each type. The identity number can be used to visualize each of these neurons using the Neuprint platform. Also listed are the number of $\alpha/\beta_p$ Kenyon cells connected to a given input neuron, as reported in Neuprint v1.0.1, as well as the percentage of input these neurons provide to the $\alpha/\beta_p$ Kenyon cells in parentheses.