Supplemental Information

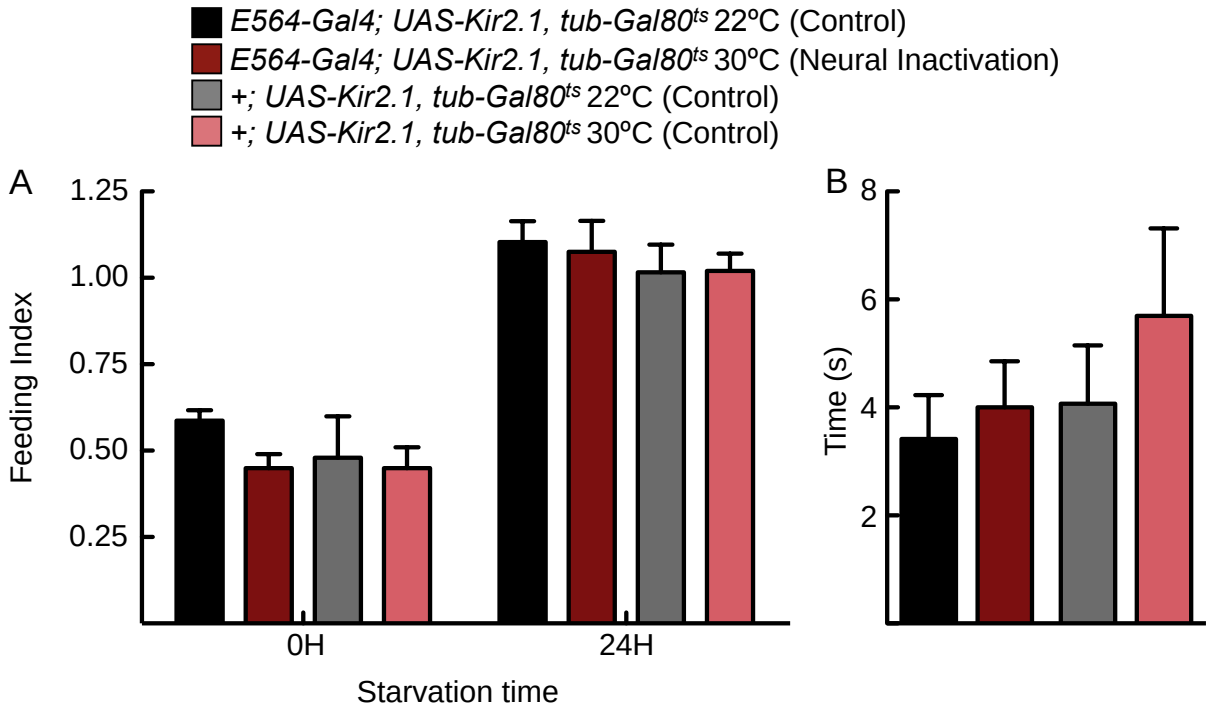

Figure S1: Inactivating E564 neurons does not alter sucrose consumption, related to Figure 1.

A. *E564-Gal4, UAS-Kir2.1, tub-Gal80^{ts}* and control flies were allowed to freely feed on 200mM sucrose spiked with blue dye for 30 minutes in both satiated (0H starvation) and deprived (24H starvation) conditions. Flies not feeding are given a score of 0, those with less than half of their abdomen full of blue dye are given a score of 1, and those with greater than half are given a score of 2. All flies consumed similar amounts of 200mM sucrose when Kir2.1 expression is induced (30ºC) or not induced (22ºC). n=3 groups of 30-60 flies each per genotype, mean±SEM, ANOVA with post-hoc Tukey test, ns.

B. The same genotypes used in A were mounted on glass slides and stimulated with 1M sucrose. Each fly was allowed to drink until it would no longer consume sucrose after 10 consecutive stimulations. Sucrose consumption time was not significantly different. n=27-36 flies per genotype, mean ± SEM, ANOVA with post-hoc Tukey test, ns.

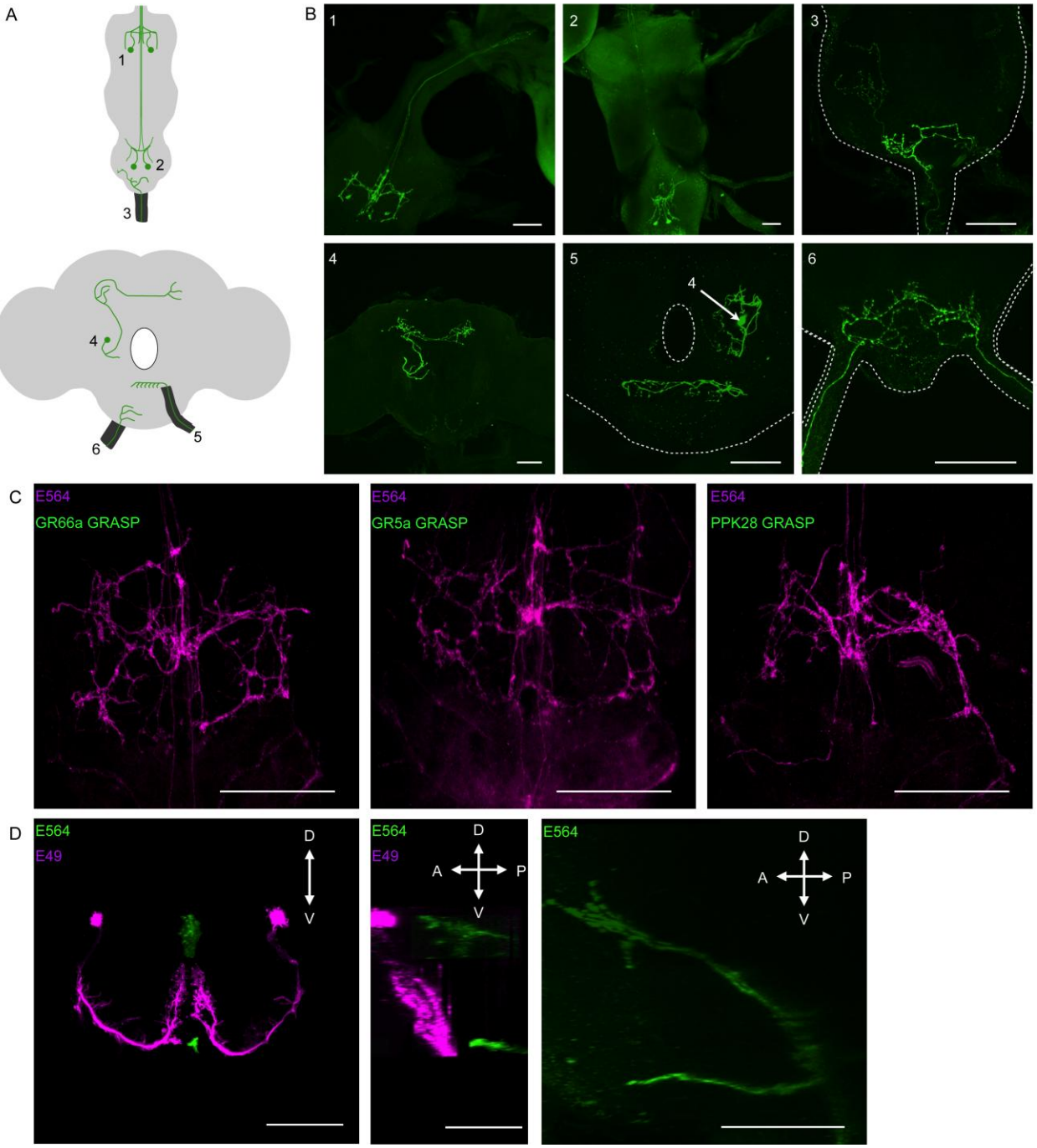

Figure S2: The 6 cell types in the *E564-Gal4* line and proximity of PER_{in} to gustatory and motor fibers, related to Figure 2.

A. Schematic of the ventral nerve cord (VNC, top) and central brain (bottom). Each cell type in the *E564-Gal4* line is illustrated in green.

B. Confocal images of each of the cell types. Each panel corresponds to the cell type number shown in A, Figure 2A-C and Figure 7C. The top 3 panels show the VNC and the bottom 3 show the brain. In panel 3, the border of the abdominal ganglion and nerve are outlined. In panel 5, the

SOG and the esophagus borders are outlined. In panel 6, the SOG and the labeller nerves are outlined. Flies were generated using the mosaic method as presented in Figure 2 and are the same genotype.

C. Contacts between gustatory dendrites and PER_{in} were examined by expressing membrane-tethered split GFP fragments in the processes, by the GRASP method as previously described (Gordon and Scott, 2009). PER_{in} is shown in magenta. No GRASP punctae (green) were observed. See Videos S1 and S2 showing 3D representations of sensory and PER_{in} projections in the ventral nerve cord.

D. Proximity of PER_{in} to motor neurons that drive proboscis extension was examined by double labeling PER_{in} and motor fibers. Cell-specific labeling was achieved by photoactivation of pa-GFP using *E564-Gal4*, *E49-Gal4*, *UAS-paGFP* flies. Left panel shows frontal view of PER_{in} in green and proboscis motor neurons in magenta. Middle panel show sagittal view and right panel shows the sagittal view of PER_{in} alone. There is no overlap in projections. Photoactivation of GFP was performed essentially as previously described (Ruta et al., 2010). Dissected brain plus ganglia preparations were secured to the bottom of a plastic dish in AHL. PER_{in} axons were photoactivated through the cervical connective, and E49 motor neurons were photoactivated at the cell body on the surface of the SOG using a 710nm two-photon laser. The anterior portion of the SOG was then imaged using a 925nm two-photon laser. Erroneous photoactivation and background was removed from the image by masking the motor neurons and PER_{in} axons. E49 motor neurons were then pseudocolored using ImageJ to enhance contrast. All scale bars are 50μm.

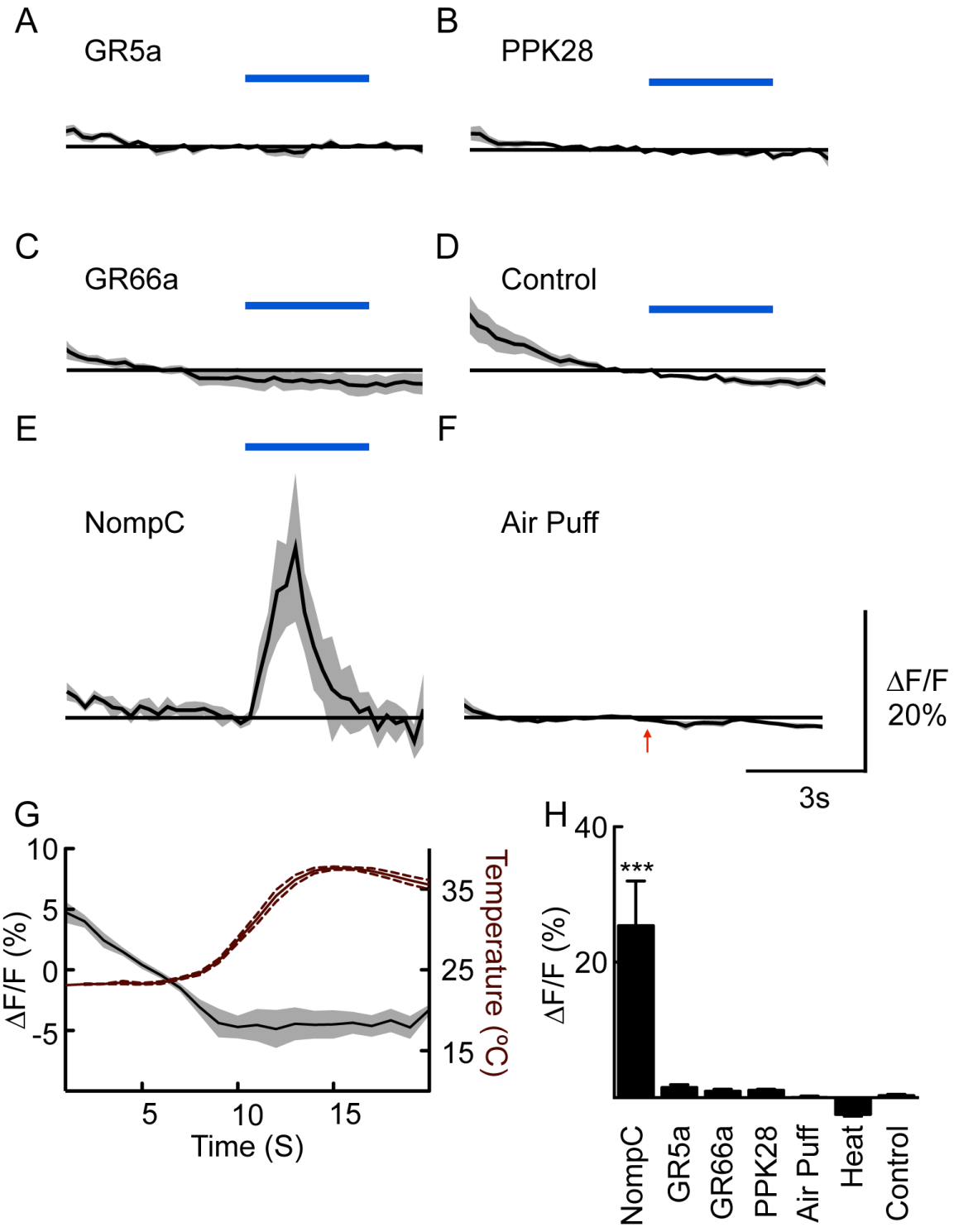
A
GR5a
B
PPK28
C
GR66a
D
Control
E
NompC
F
Air Puff
ΔF/F
20%
3s
G
10
5
0
-5
ΔF/F (%)
35
25
15
Temperature (°C)
5
10
15
Time (S)
H
40
20
***
ΔF/F (%)
NompC
GR5a
GR66a
PPK28
Air Puff
Heat
Control

Figure S3: PER_{in} neurons do not respond to channelrhodopsin-2 (ChR2)-mediated stimulation of gustatory neurons, related to Figure 4.

A-E. Stimulation of sugar-sensing (A), water-sensing (B), bitter-sensing (C), and no (D) sensory neurons while monitoring GCaMP3 responses in PER_{in} dendrites. Flies were prepared in an identical manner to those in NompC-ChR2 experiments (data from Figure 4D is shown as a reference in panel E), with identical light stimulation (blue bar).

F. A gentle puff of air (red arrow) was delivered to legs while monitoring GCaMP3 responses in PER_{in} dendrites. Air was delivered manually through a glass capillary.

G. Leg sensory neurons were stimulated using a heat probe to activate thermosensitive neurons. ΔF/F values are shown in gray (left axis), and temperature values of the heat probe are shown in red (right axis).

H. Summary data of maximum ΔF/F for each stimulation. n=6-8 flies per genotype, mean±SEM, ANOVA with post-hoc Tukey test, ***P<0.001.

For GCaMP3 traces, lines and shaded areas represent mean±SEM. Flies used were *UAS-GCaMP3; Sensory-LexA* (GR5a, PPK28, GR66a)/*E564-Gal4*, *UAS-GCaMP3*; *LexAop-ChR2, UAS-GCaMP3*. Control flies used lacked a sensory LexA driver.

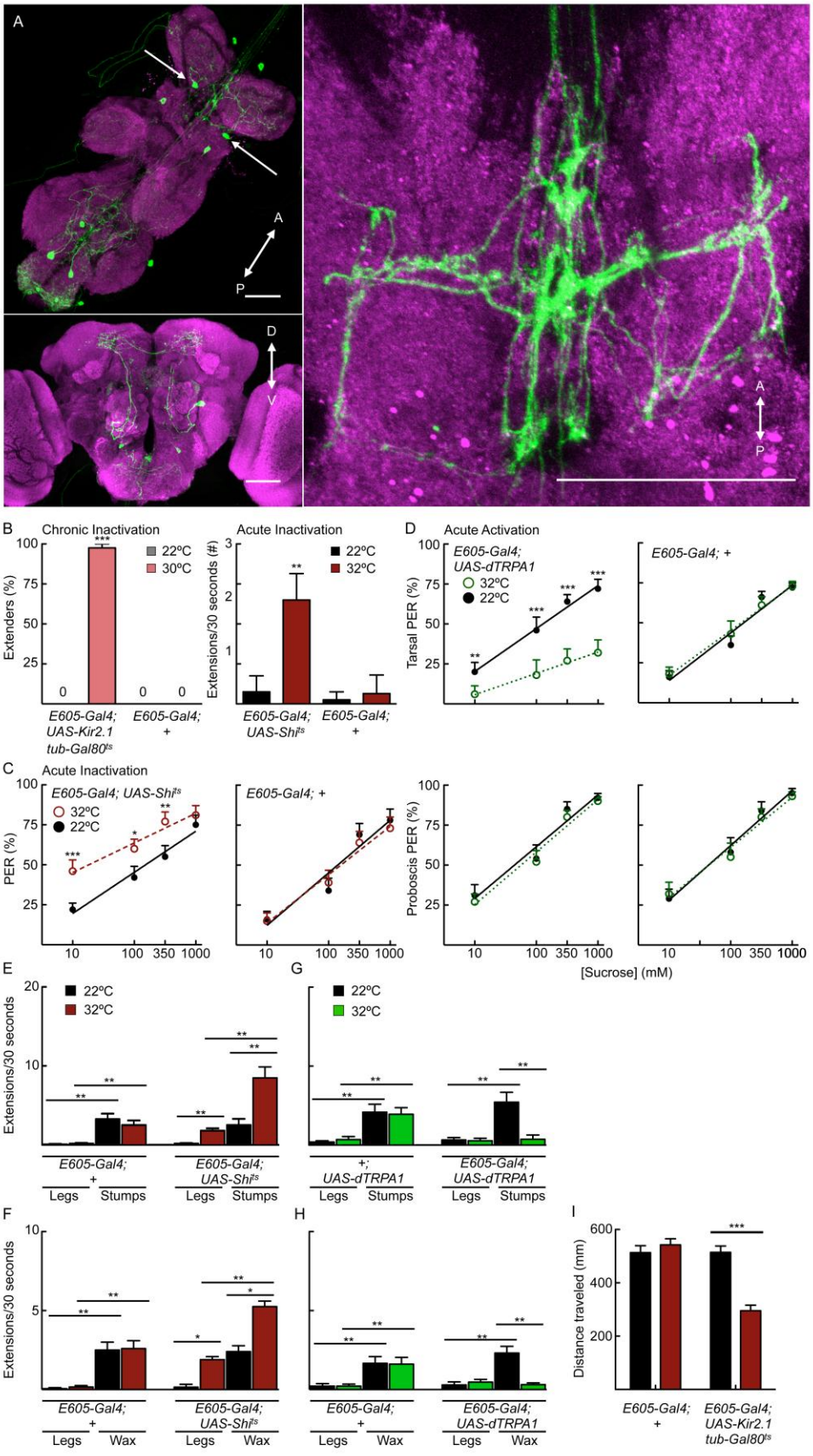
A
A
P
D
V
A
P
B
Chronic Inactivation
Extenders (%)
22ºC
30ºC
E605-Gal4; UAS-Kir2.1 tub-Gal80ts
E605-Gal4; +
Acute Inactivation
Extensions/30 seconds (#)
22ºC
32ºC
E605-Gal4; UAS-Shits
E605-Gal4; +
D
Acute Activation
E605-Gal4; UAS-dTRPA1
32ºC
22ºC
Tarsal PER (%)
E605-Gal4; +
C
Acute Inactivation
E605-Gal4; UAS-Shits
32ºC
22ºC
PER (%)
E605-Gal4; +
Proboscis PER (%)
[Sucrose] (mM)
E
Extensions/30 seconds
22ºC
32ºC
E605-Gal4; +
E605-Gal4; UAS-Shits
Legs
Stumps
G
22ºC
32ºC
+; UAS-dTRPA1
E605-Gal4; UAS-dTRPA1
Legs
Stumps
F
Extensions/30 seconds
E605-Gal4; +
E605-Gal4; UAS-Shits
Legs
Wax
H
E605-Gal4; +
E605-Gal4; UAS-dTRPA1
Legs
Wax
I
Distance traveled (mm)
E605-Gal4; +
E605-Gal4; UAS-Kir2.1 tub-Gal80ts

Figure S4. A second Gal4 line, *E605-Gal4*, containing PER_{in} neurons displays similar behavioral phenotypes, related to Figure 7.

A. Expression of *UAS-CD8-GFP* in *E605-Gal4* neurons in VNC (top left) and central brain (bottom left). PER_{in} is labeled (top left, arrows) and dendrites in VNC are shown (right). Scale bars are 50μm.

B. Chronically silencing neurons in *E605-Gal4; UAS-Kir, tub-Gal80^{ts}* (left) produced constitutive proboscis extension in nearly 100% of animals, a phenotype not observed in *E605-Gal4* flies and non-induced flies (*E605-Gal4; UAS-Kir, tub-Gal80^{ts}* , 22°C). 0 indicates that no flies showed proboscis extension. n=20-25 flies/genotype, mean±95%CI, Fisher's exact test, *** P<0.001. Acutely silencing neurons in *E605-Gal4; UAS-Shi^{ts}* flies (right) increased spontaneous proboscis extensions (32°C, red bars) compared to genetic controls at permissive temperature (22°C, black bars) or *E605-Gal4* controls. n=24 flies, mean±SEM, student's t-test, **P<0.01.

C. Proboscis extension response to sucrose (10-1000mM) in *E605-Gal4, UAS-Shi^{ts}* (left) and *E605-Gal4* flies (right) at permissive (black) and restrictive temperatures (red). n=30 flies, mean±95% CI, Fisher's exact test, ***P<0.001, **P<0.01, *P<0.05.

D. Proboscis extension response to tarsal (top) or proboscis (bottom) stimulation in *E605-Gal4, UAS-dTRPA1* (left) and *E605-Gal4* control flies (right) at 22°C (black) and 32°C (green). n=30 flies/condition, mean±95% CI, Fisher's exact test, ***P<0.001, **P<0.01.

E. *E605-Gal4; UAS-Shi^{ts}* flies and *E605-Gal4* or *UAS-Shi^{ts}* flies with or without legs (legs vs. stumps) at permissive (22°C, black bars) and restrictive (32°C, red bars) temperatures. Removal of legs in control flies caused an increase in spontaneous proboscis extensions, which was similar at both temperatures. Removal of legs in *E605-Gal4, UAS-Shi^{ts}* flies also increased spontaneous extensions, and was greatly enhanced at restrictive temperature. n=15-19 flies/condition, mean±95% CI, Fisher's exact test, ***P<0.001, **P<0.01.

F. Fly legs were immobilized with wax and proboscis extensions were examined at permissive (black bars) and restrictive (red bars) temperatures. n=16-19 flies, mean±95% CI, Fisher's exact test, **P<0.01, *P<0.05.

G. *UAS-dTRPA1* controls and *E605-Gal4; UAS-dTRPA1* flies with or without legs (legs vs. stumps) at 22ºC (black) and 32ºC (green). Removal of legs in *E605-Gal4, UAS-dTRPA1* flies increased spontaneous extensions, but this effect was abolished at 32ºC upon dTRPA1 activation. n=14-19 flies, mean±95% CI, Fisher's exact test, **P<0.01.

H. Activating E605 neurons abolished the increase in spontaneous proboscis extensions caused by leg immobilization (wax). n=17-20 flies/condition, all data is mean±SEM, ANOVA with Tukey post-hoc test, **P<0.01.

I. *E605-Gal4; UAS-Kir2.1, tub-Gal80^{ts}* flies show reduced movement when compared to *E605-Gal4; UAS-Kir2.1, tub-Gal80^{ts}* controls at 22°C or *E605-Gal4* controls. n=20flies/condition, mean±SEM, ANOVA with Tukey post hoc test, ***P<0.001.

Video S1. Comparison of PER_{in} and sugar sensory projections, related to Figure S2.
Ventral nerve cord showing PER_{in} (green) and sugar gustatory axons (red). To generate 3d comparisons between PER_{in} dendrites and sensory axons, confocal stacks of *E564-Gal4, GR5a-Gal4, UAS-GFP* flies were generated. PER_{in} dendrites and gustatory sensory neuron axons were masked and pseudo-colored (ImageJ) to remove the other cell types found in E564 and allow for easier comparisons. Video is 225x225x82µm.

Video S2. Comparison of PER_{in} and bitter sensory projections, related to Figure S2.
Ventral nerve cord showing PER_{in} (green) and bitter gustatory axons (red). To generate 3d comparisons between PER_{in} dendrites and sensory axons, confocal stacks of *E564-Gal4, GR66a-Gal4, UAS-GFP* flies were generated. PER_{in} dendrites and gustatory sensory neuron axons were masked and pseudo-colored (ImageJ) to remove the other cell types found in E564 and allow for easier comparisons. Video is 225x225x64µm.