Neuron

Supplemental Information

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SUPPLEMENTARY INFORMATION

- **Supplemental Figure Legends (7 figures)**
- **Supplemental Movie Legends (6 movies)**
- **Supplemental Experimental Procedures**
- **Supplemental References**

Figure S1

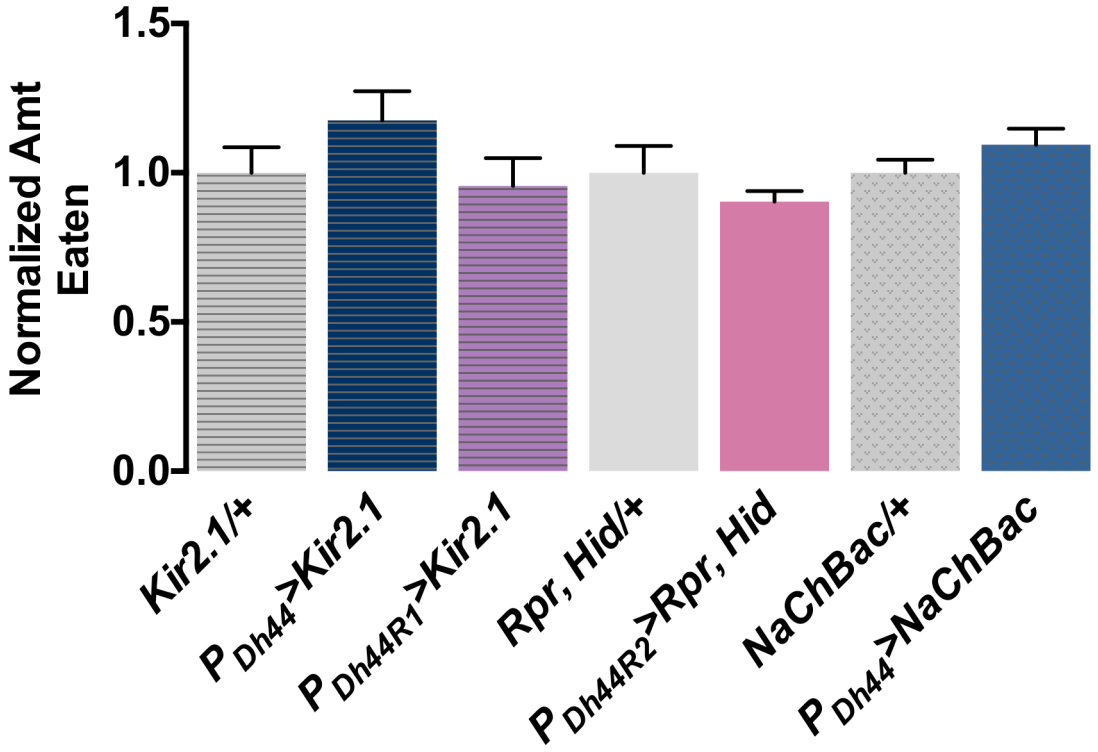

Normalized Amt Eaten
1.5
1.0
0.5
0.0
Kir2.1/+
P Dh44>Kir2.1
P Dh44R1>Kir2.1
Rpr, Hid/+
P Dh44R2>Rpr, Hid
NaChBac/+
P Dh44>NaChBac

Figure S2

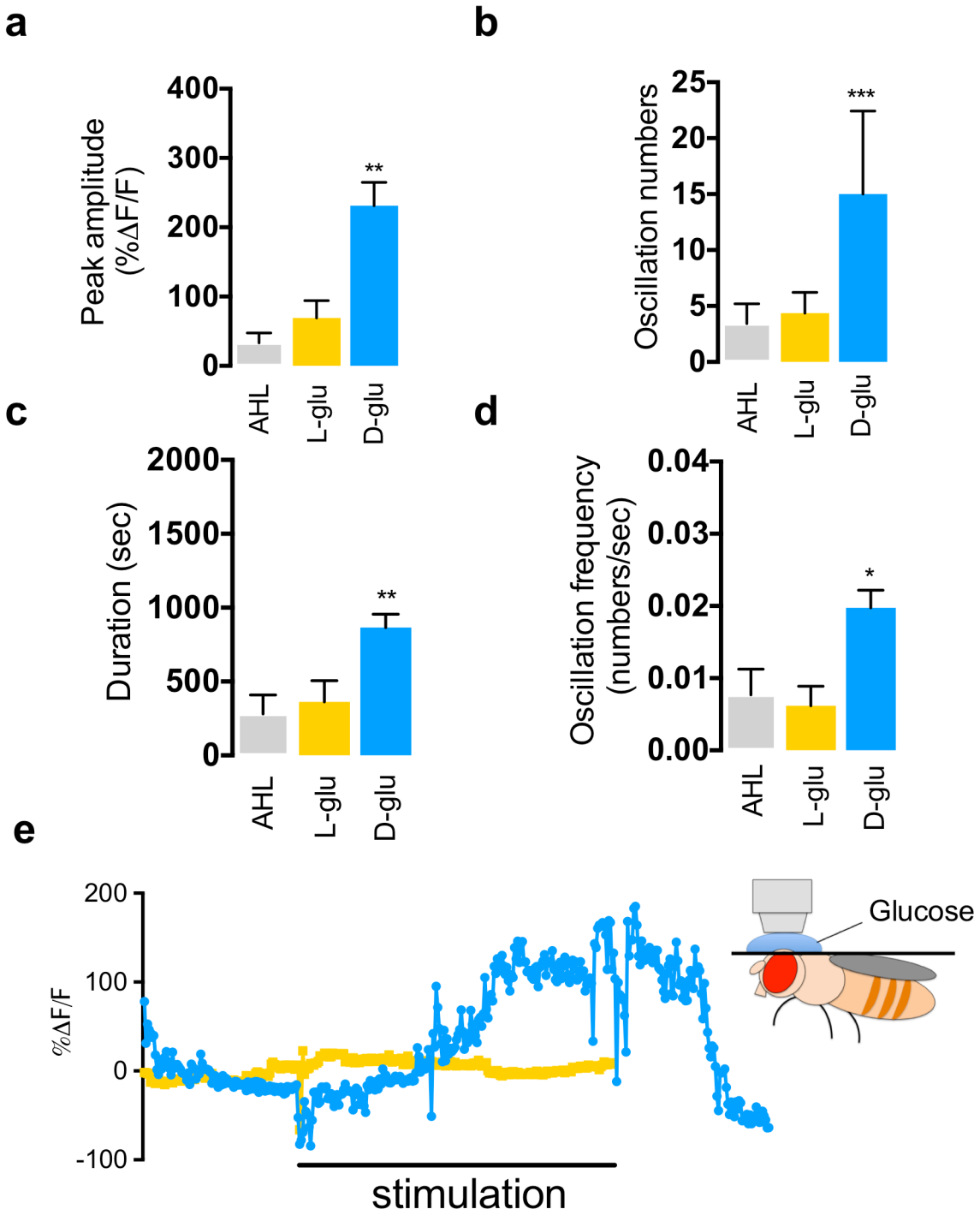

a
Peak amplitude (%ΔF/F)
400
300
200
100
0
**
AHL
L-glu
D-glu
b
Oscillation numbers
25
20
15
10
5
0
***
AHL
L-glu
D-glu
c
Duration (sec)
2000
1500
1000
500
0
**
AHL
L-glu
D-glu
d
Oscillation frequency (numbers/sec)
0.04
0.03
0.02
0.01
0.00
*
AHL
L-glu
D-glu
e
%ΔF/F
200
100
0
-100
stimulation
Glucose

Figure S3

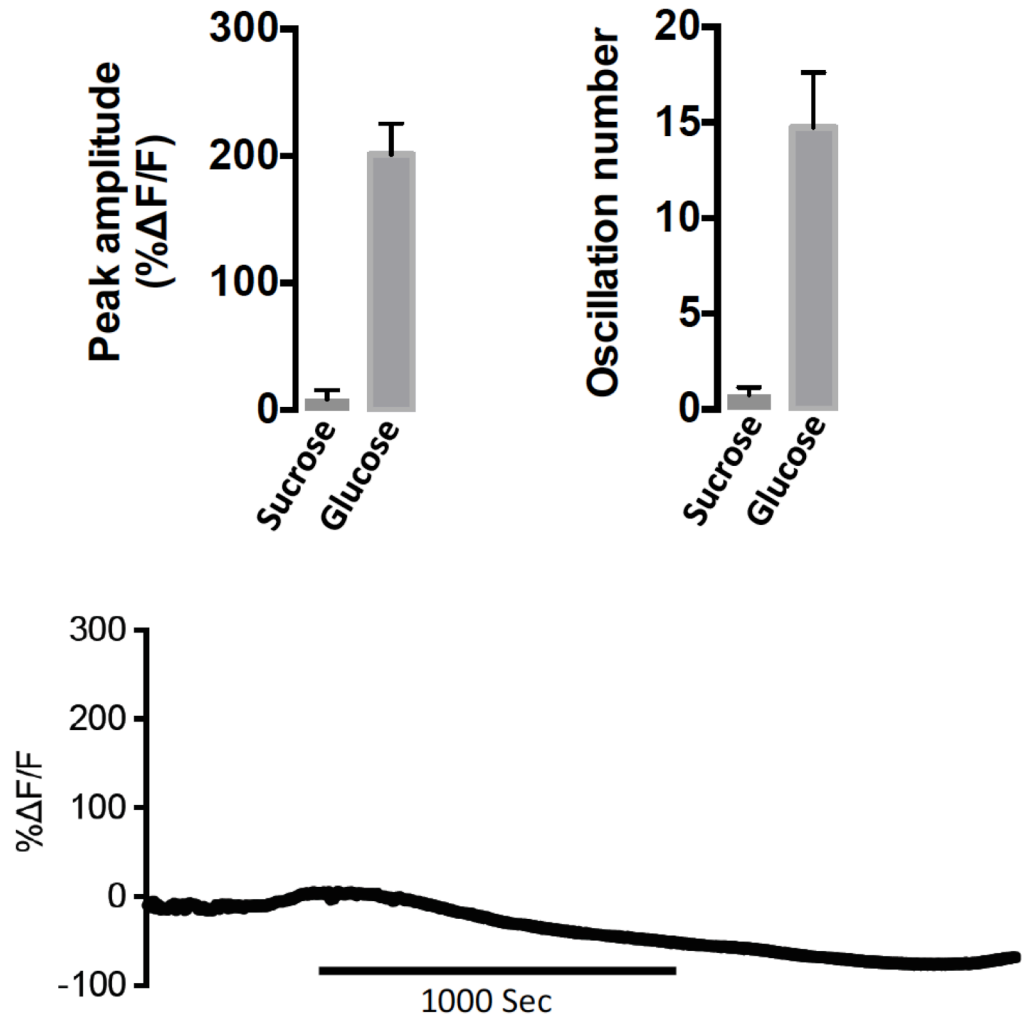

Peak amplitude (%ΔF/F)
300
200
100
0
Sucrose
Glucose
Oscillation number
20
15
10
5
0
Sucrose
Glucose
%ΔF/F
300
200
100
0
-100
1000 Sec

Figure S4

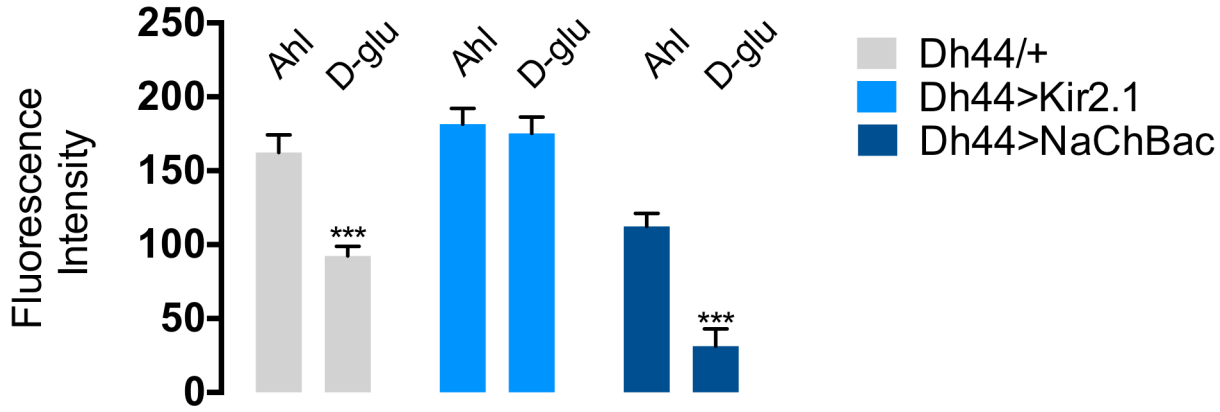

250
200
150
100
50
0
Fluorescence Intensity
Ahl
D-glu
Ahl
D-glu
Ahl
D-glu
***
***
Dh44/+
Dh44>Kir2.1
Dh44>NaChBac

Figure S5

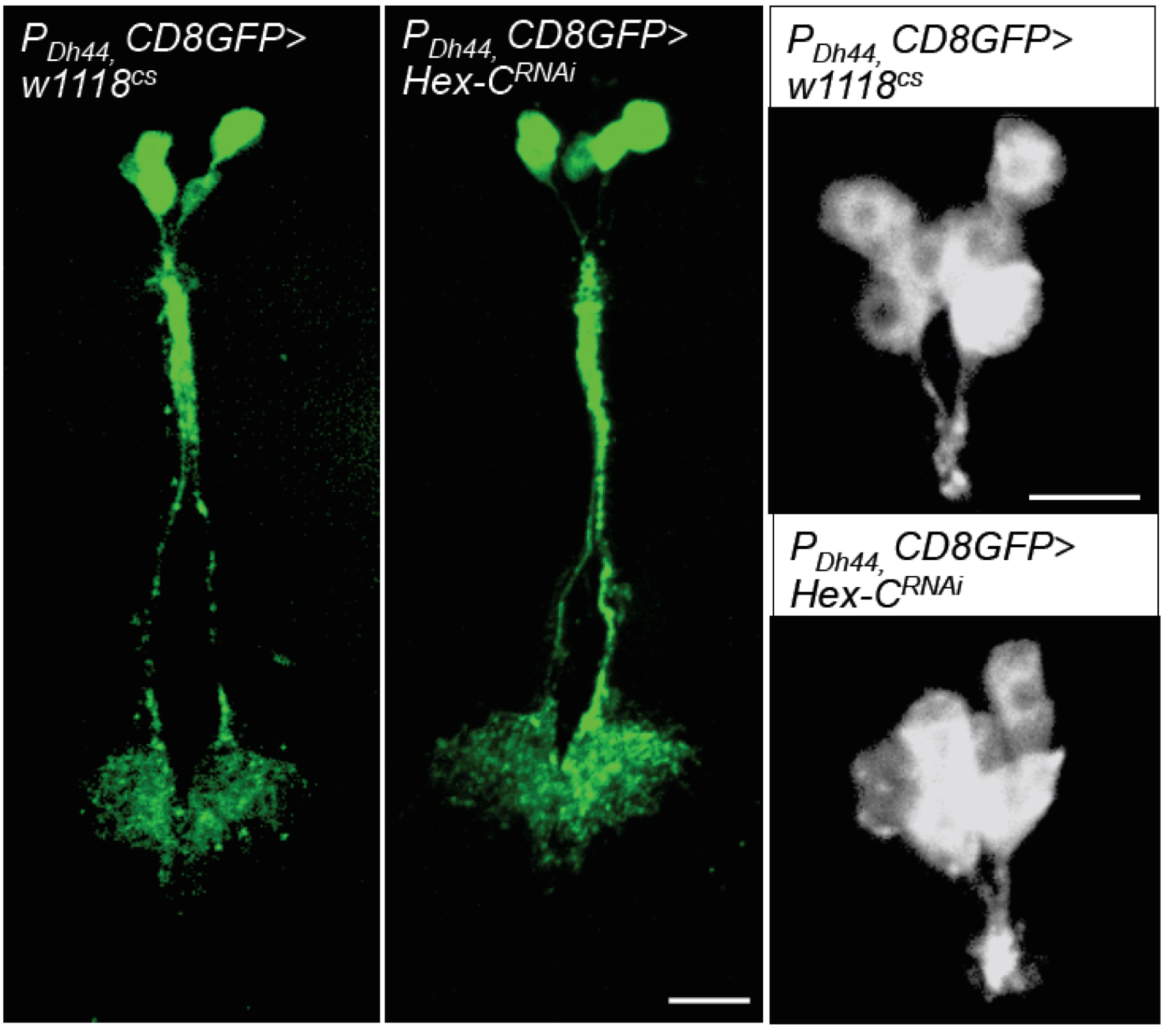

$P_{Dh44,}$ CD8GFP>
$w1118^{cs}$
$P_{Dh44,}$ CD8GFP>
$Hex\text{-}C^{RNAi}$
$P_{Dh44,}$ CD8GFP>
$w1118^{cs}$
$P_{Dh44,}$ CD8GFP>
$Hex\text{-}C^{RNAi}$

Figure S6

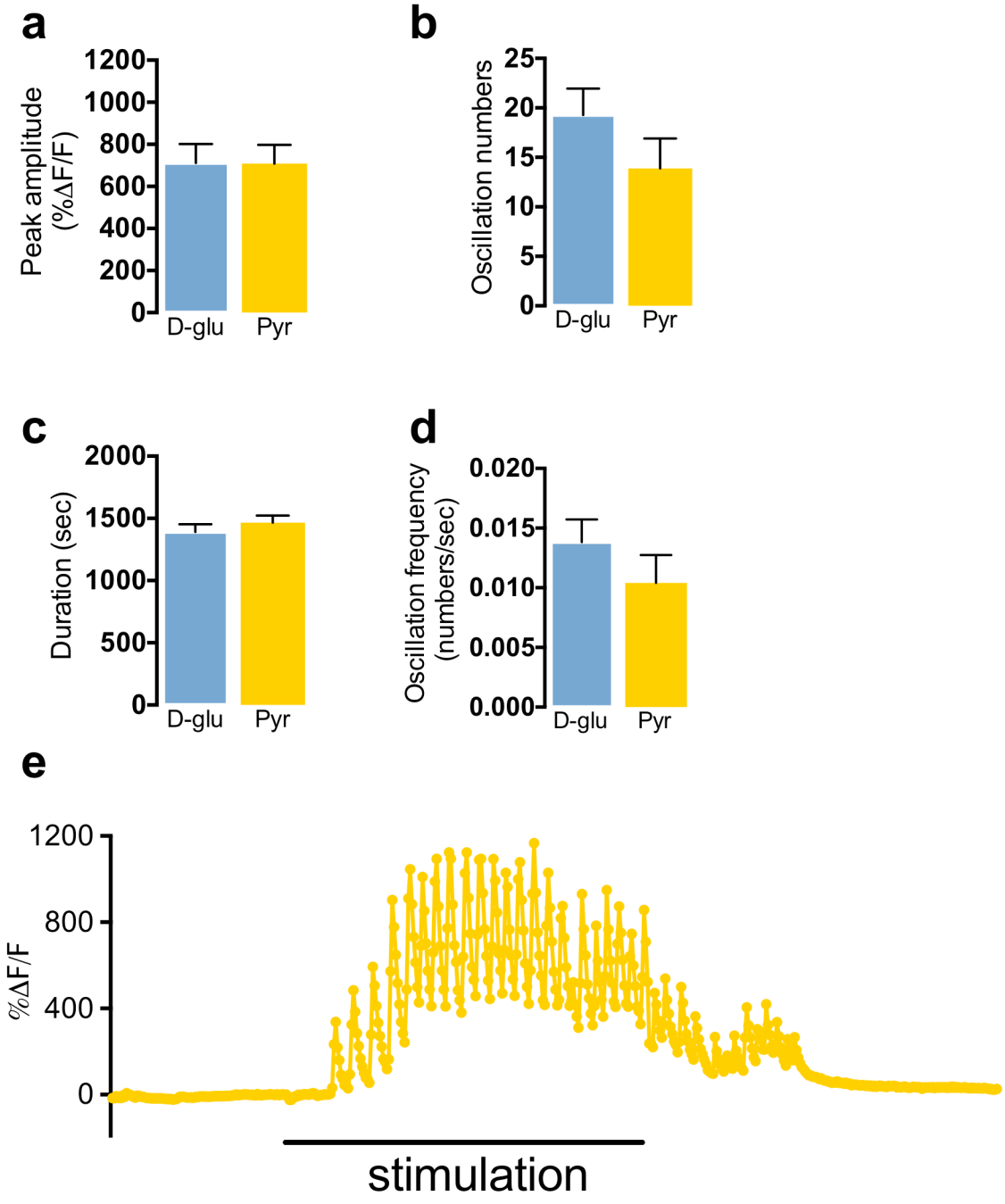
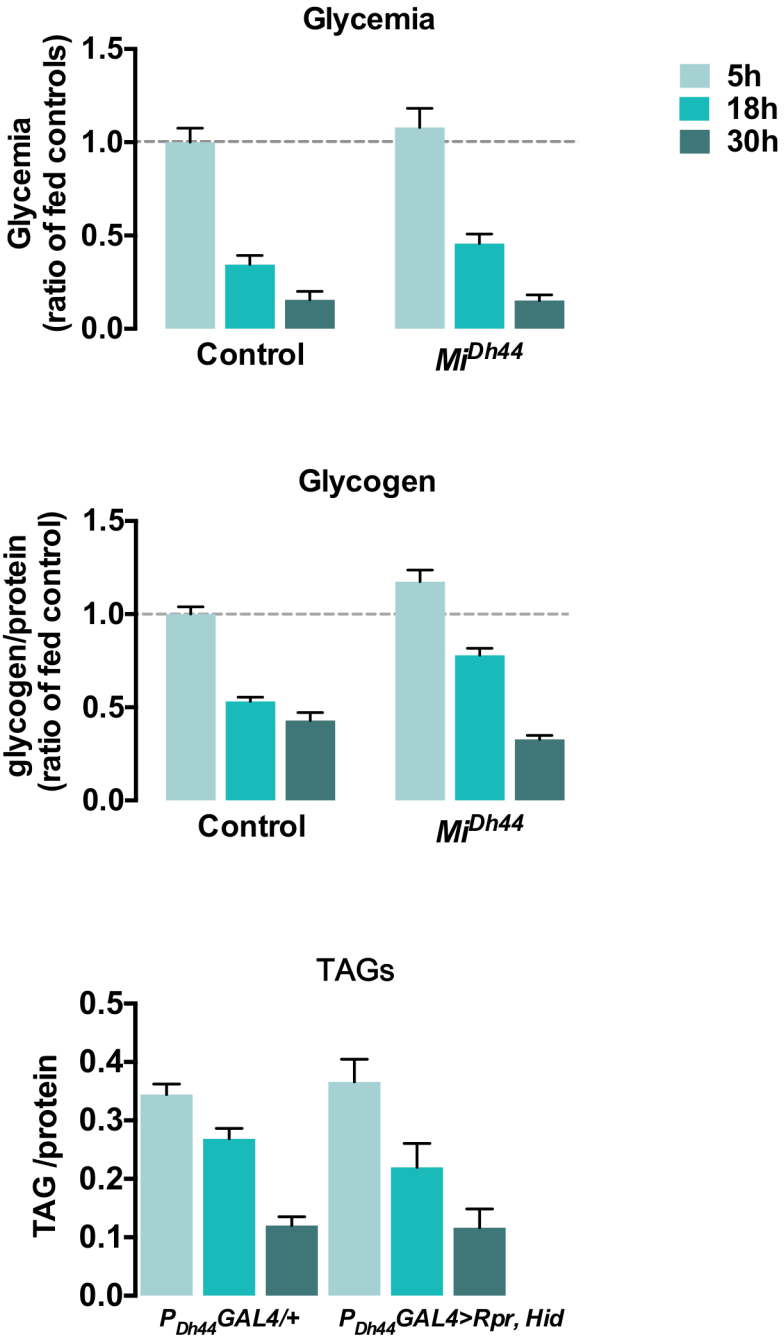

a
Peak amplitude (%ΔF/F)
1200
1000
800
600
400
200
0
D-glu
Pyr
b
Oscillation numbers
25
20
15
10
5
0
D-glu
Pyr
c
Duration (sec)
2000
1500
1000
500
0
D-glu
Pyr
d
Oscillation frequency (numbers/sec)
0.020
0.015
0.010
0.005
0.000
D-glu
Pyr
e
%ΔF/F
1200
800
400
0
stimulation

Figure S7


Glycemia
Glycemia (ratio of fed controls)
1.5
1.0
0.5
0.0
Control
MiDh44
5h
18h
30h
Glycogen
glycogen/protein (ratio of fed control)
1.5
1.0
0.5
0.0
Control
MiDh44
TAGs
TAG /protein
0.5
0.4
0.3
0.2
0.1
0.0
PDh44GAL4/+
PDh44GAL4>Rpr, Hid

Supplementary Figure Legends

SI1. Manipulating the function of Dh44 neurons and their receptors does not have an effect on the amount of food intake, related to Figure 1.

The amounts of food consumed by 18h-starved flies of the described genotypes for 30 minutes. The food comprises 50% fly food + 50mM D-glucose containing 0.05% blue dye (erioglaucine). The data is normalized to each control. n=3-9. Error bars, s.e.m.

SI2. **Dh44 neurons *in vivo* respond to D-glucose, related to Figure 3.**

a-d) The a) amplitude, b) oscillation number, c) duration, and d) oscillation frequency of Dh44 neurons expressing UAS-GCaMP6.0 in response to AHL (grey), 100mM L-glucose (yellow, L-glu) or 100mM D-glucose (blue, D-glu). n=7-19. Error bars are s.e.m.

e) A representative trace showing the activity of Dh44 neurons to 100mM D-glucose but not 100mM L-glucose. Prior to glucose stimulation, Dh44 neurons have spontaneous oscillations with low amplitude. *Right*, a schematic illustrating the *in vivo* imaging setup: a small part of cuticle was removed from the top of the fly head to allow visualization of Dh44 neurons.

SI3. Sucrose does not activate Dh44 neurons, related to Figure 3.

Top. The change in peak amplitude (%ΔF/F) and oscillation numbers as a measure of neural activity in the brains of P_{Dh44}*>GCaMP3.0* flies that are exposed to sugar free AHL saline followed by AHL containing 20mM sucrose or AHL containing 80mM glucose. No calcium transient was observed when the brains were stimulated by sucrose; see Figure 3B for comparison. N=8-12. Error bars are s.e.m. *Bottom*. A representative trace of Dh44 neuronal response to AHL containing 20mM sucrose.

SI4. Manipulations of Dh44 neuronal activity have effects on the release of the Dh44 neuropeptide, related to Figure 3.

The brains of flies carrying Dh44-Gal4; UAS-Kir2.1 (light blue bar), flies carrying Dh44-GAL4; UAS-NaChBac (dark blue bar) and flies carrying Dh44-GAL4 as controls (gray bar) were stimulated with AHL containing 80mM D-glucose or the osmolarity-balanced AHL. Then these brains were fixed and stained with anti-Dh44 antibody. The amount of Dh44 peptide present inside the Dh44 cells was calculated as a measure of the cellular fluorescence (fluorescent intensity, y-axis). Stimulating the brains of control flies with D-glucose resulted in the release of approximately 50% of the Dh44 peptide (gray bars). Stimulating the brains of P_{Dh44}*>Kir2.1* flies with D-glucose did not result in the neuropeptide release (light blue bars). By contrast, the brains of P_{Dh44}*>NaChBac* flies released Dh44 neuropeptide even without glucose stimulation and released nearly all Dh44 upon stimulation (dark blue bars). n=6-21. ****P<0.001.* Error bars are s.e.m

SI5. *Hexokinase-C* knock-down does not affect the cellular integrity of Dh44 neurons, related to Figure 3.

Confocal z-stack images illustrating the morphology of Dh44 neurons labeled by a CD8GFP transgene. The dendritic processes of Dh44 neurons with (*middle*) and without (*left*) the expression of *Hex-C* RNAi. Their cell bodies with (*right, bottom*) and without (*right, top*) the expression of *Hex-C* RNAi. The morphology of Dh44 neurons with and without *Hex-C* knockdown is grossly indistinguishable. Scale bars, 20μm.

SI6. The activity of Dh44 neurons is stimulated by pyruvate, related to Figure 3.

a) The amplitude, **b)** oscillation number, **c)** duration, and **d)** oscillation frequency of Dh44 neurons of flies expressing Dh44>GCaMP6.0 in response to 20mM D-glucose (blue, D-glu) or 20mM pyruvate (yellow, Pyr). n=11-17. Error bars are s.e.m.

e) A representative trace illustrating the response of Dh44 neurons to 20mM pyruvate.

SI7. The levels of hemolymph glycemia, glycogen and triglyceride are normal in *Dh44* mutants or flies in which Dh44 neurons are ablated, related to Figure 4.

The levels of circulating glucose and trehalose (*top*) and glycogen stores (*middle*) in $w1118^{CS}$ and Mi^{Dh44} mutant were measured at different time points during food deprivation (5h, 18h, 30h). Triglyceride contents- TAGs (*bottom*) were also measured using flies in which Dh44 neurons are ablated. The data were normalized to the 5h control. n=12-15. Error bars, s.e.m.

Supplementary Movie Legends

S1. Exposure of the brain to nutritive D-glucose activates Dh44 neurons in flies carrying P_{Dh44}-GAL4 and UAS-GCaMP3.0, related to Figure 3.

S2. Artificial activation of Dh44R1 neurons in P_{Dh44R1}-*GAL4>TrpA1* flies results in an increased rate of proboscis extension, related to Figure 5.
P_{Dh44R1}-*GAL4>TrpA1* flies were introduced into a glass Pasteur pipette at 30°C and filmed with a high-speed camera at 2 frames/second for 1 minute. The video was processed at 15 frames/second.

S3. The control AHL has no effect on the gut motility in wild type flies, related to Figure 6.
The gut of a 18h-starved $w1118^{CS}$ fly was dissected in AHL, pinned to a sylgard plate and imaged in AHL. The accelerated video shows minutes 1-5' during the control AHL incubation. Movies shown in S3 – S6 were accelerated 3X for easy visualization.

S4. Dh44 peptide stimulates the gut motility in wild type flies, related to Figure 6. The gut of a 18h-starved $w1118^{CS}$ fly was dissected in AHL, pinned to a sylgard plate and imaged. After 5 minutes in AHL (Movie S3), the same gut was perfused with AHL containing Dh44 peptide and then imaged for 5 additional minutes. The accelerated video shows the activity of the gut during the Dh44 peptide incubation (6-10'). Arrows depict increased gut movements.

S5. The control AHL has no effect on the gut motility of Mi^{Dh44R2} mutant, related to Figure 6. The gut of a 18h-starved Mi^{Dh44R2} mutant was dissected in AHL, pinned to a sylgard plate and imaged in AHL. The accelerated video shows minutes 1-5' during the control AHL incubation.

S6. Dh44 peptide has no effect on the gut motility of *Mi^{Dh44R2}* mutant, related to Figure 6.

The gut of a 18h-starved *Mi^{Dh44R2}* mutant was dissected in AHL, pinned to a sylgard plate and imaged. After 5 minutes in AHL (Movie S5), the same gut was perfused with AHL containing Dh44 peptide and then imaged for 5 additional minutes. The accelerated video shows the activity of the gut during the Dh44 peptide incubation (6-10').

Supplementary Experimental Procedures

Measurement of food intake

After 18h-starved 30 male flies were fed with colored food (50% fly food + 50mM D-glucose + 0.05% erioglaucine) for 30 minutes, they were flash-frozen, homogenized in 1ml of PBS, and spun down on a tabletop centrifuge at the maximum speed for 10 minutes. 100µl of the supernatant was transferred to a plate reader and the light absorbance was measured at 625nm. Background absorbance from flies that were fed the same food without the dye was subtracted from each measurement. The amount of food eaten by groups of flies was linearly regressed from known standards.

Glycogen and triglyceride measurements

Glycogen and triglyceride were measured as previously described (Dus et al., 2011).

In vivo calcium imaging

For *in vivo* imaging, a fly was immobilized by heated wax on a plastic plate that exposes a small part of the fly head to AHL. A small window on the top of the head was surgically removed using a fine needle tip and forceps, and fat and tracheae were subsequently removed to completely expose the brain to saline (Ai et al., 2010). Sugar solution (100mM glucose + AHL) was delivered at the 100th frame after the start of the experiment and remained for next 200 frames, followed by AHL washout.

Supplemental References

Ai, M., Min, S., Grosjean, Y., Leblanc, C., Bell, R., Benton, R., and Suh, G.S. (2010). Acid sensing by the Drosophila olfactory system. Nature *468*, 691-695.

Dus, M., Ai, M., and Suh, G.S. (2013). Taste-independent nutrient selection is mediated by a brain-specific Na+ /solute co-transporter in Drosophila. Nature neuroscience *16*, 526-528.

Dus, M., Min, S., Keene, A.C., Lee, G.Y., and Suh, G.S. (2011). Taste-independent detection of the caloric content of sugar in Drosophila. Proceedings of the National Academy of Sciences of the United States of America *108*, 11644-11649.