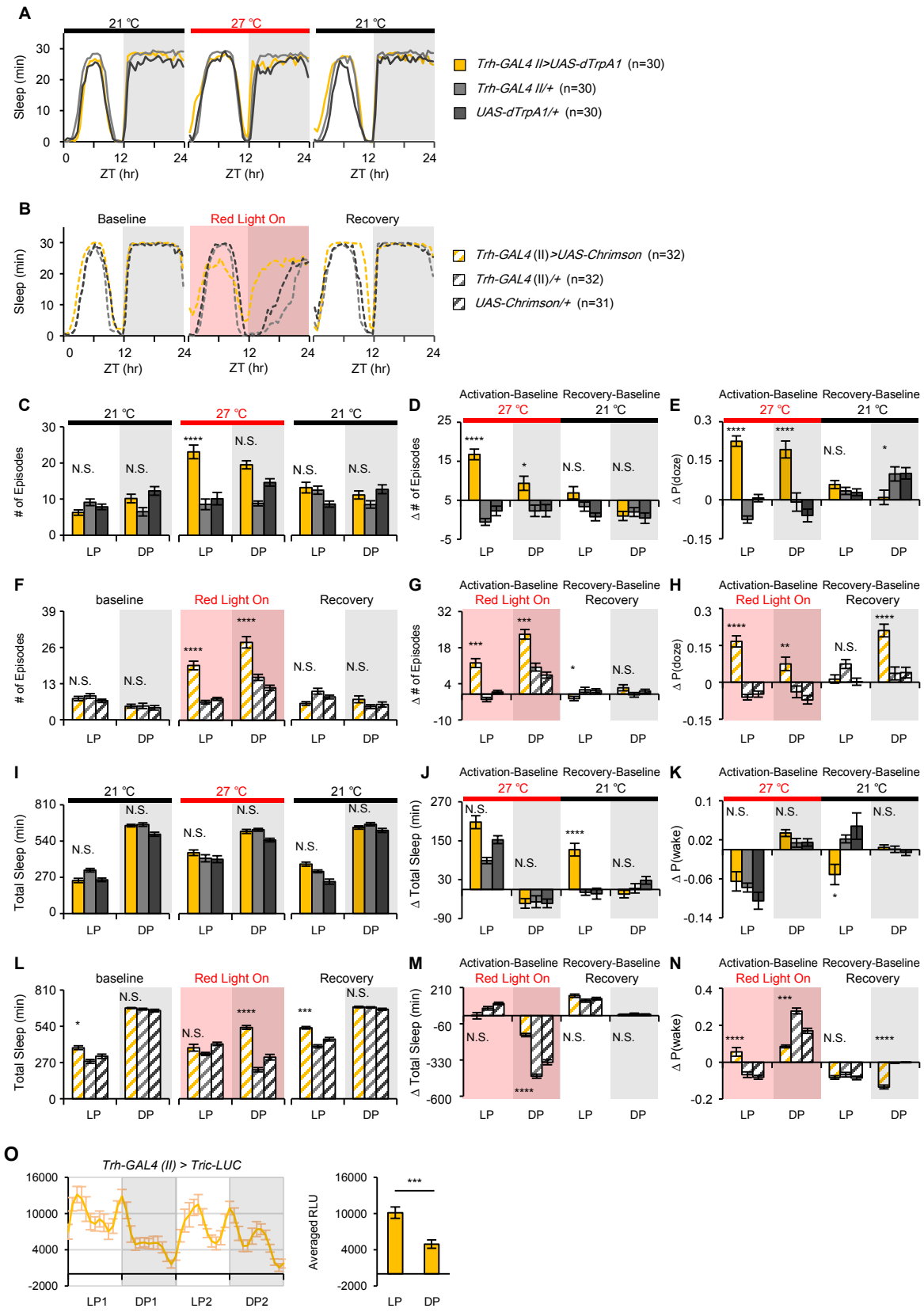
A
21 ℃
27 ℃
21 ℃
Sleep (min)
ZT (hr)
Trh-GAL4 II>UAS-dTrpA1 (n=30)
Trh-GAL4 II/+ (n=30)
UAS-dTrpA1/+ (n=30)
B
Baseline
Red Light On
Recovery
Trh-GAL4 (II)>UAS-Chrimson (n=32)
Trh-GAL4 (II)/+ (n=32)
UAS-Chrimson/+ (n=31)
C
# of Episodes
N.S.
D
Activation-Baseline
Recovery-Baseline
Δ # of Episodes
E
Δ P(doze)
F
baseline
G
H
I
Total Sleep (min)
J
Δ Total Sleep (min)
K
Δ P(wake)
L
M
N
LP
DP
O
Trh-GAL4 (II) > Tric-LUC
LP1
DP1
LP2
DP2
Averaged RLU

Figure S1. Activation of an independent *Trh-GAL4* causes sleep fragmentation without loss of total sleep, Related to Figure 1 and 2. Movement data captured in 1 min bins were used to extract sleep using the standard definition of a sleep bout as 5 min of inactivity [13]. Sleep profiles of baseline, activation and recovery days. The experimental genotypes, *Trh-GAL4 (II)>dTrpA1* (A) and *Trh-GAL4(II)>Chrimson* (B), are shown in gold, *UAS/+* and *GAL4/+* controls are in gray. Activation of *Trh-GAL4(II)* cells increases the number of sleep episodes (C,D,F,G). The change from baseline day (in minutes) is shown for the day of activation and the recovery day. P(doze), the probability of transitioning from a wake state to a sleep state (Wiggin et al., 2019), is increased during activation of *Trh-GAL4(II)+* neurons (E,H). Effect is shown as change from baseline day. Heat activation has no significant effect on the amount of sleep compared to controls (I), while light activation causes an increase in sleep during the night (L). The change in total sleep time (minutes) compared to the baseline day for activation and recovery days shows that heat (G) has no significant effects on total sleep on the activation day, but that total sleep increases on the recovery day, while light actually decreases sleep relative to controls at night (J). Heat at night did not change P(wake) relative to both baseline day and controls, while light had significant effects in both night and day on this parameter (H and K). *Trh-GAL4 (II)+* neurons exhibit a rhythmic daily activity pattern as visualized with a Tric-LUC reporter (O). LP: light period; DP: dark period. ZT: Zeitgeber Time. Asterisks in the figure indicate significant differences between the experimental genotype and both GAL4 and UAS controls. *$p<0.05$; **$p<0.01$; *** $p<0.001$; **** $p<0.0001$; N.S.: not significant.

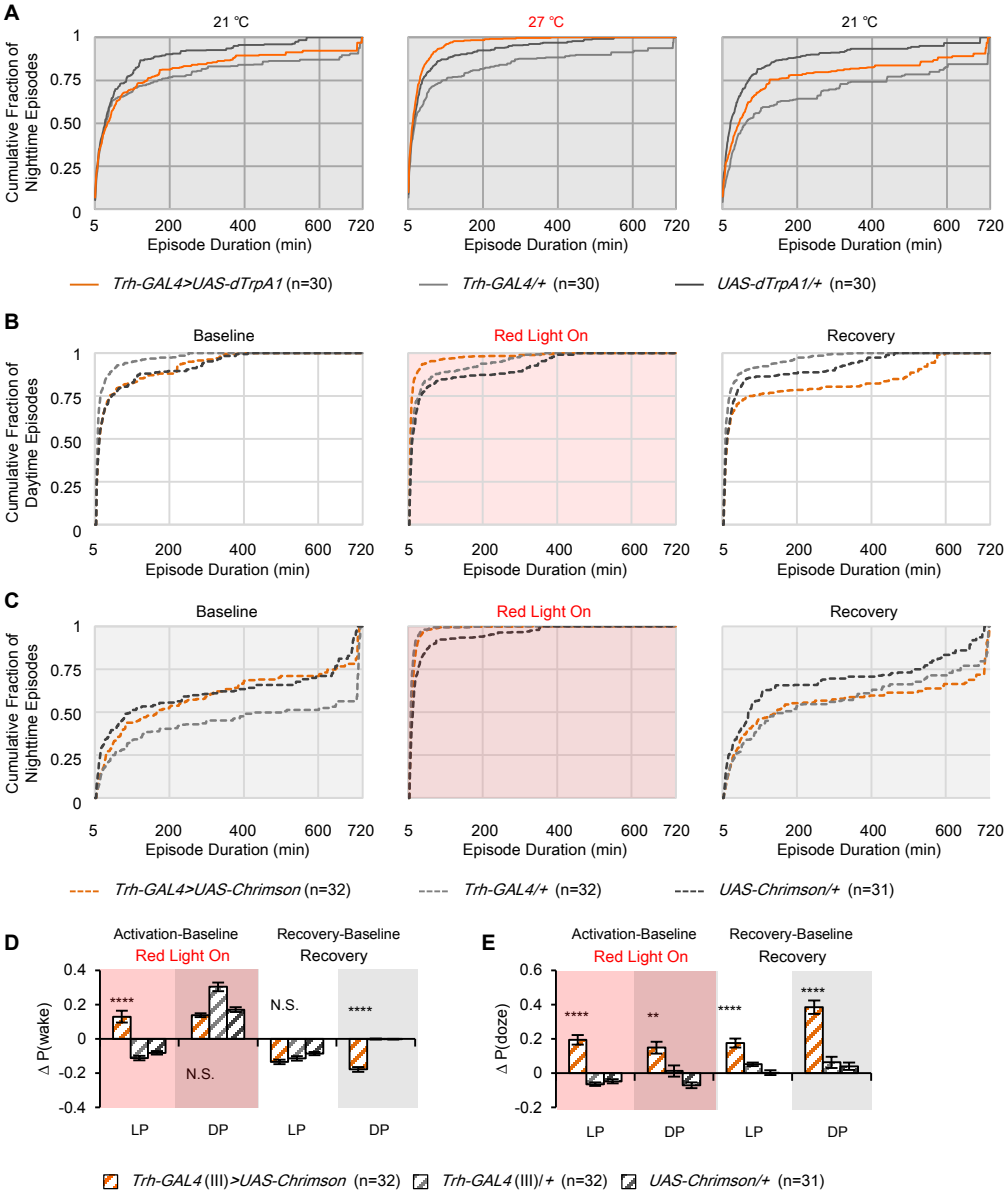
A
21 ℃
27 ℃
21 ℃
Cumulative Fraction of Nighttime Episodes
Episode Duration (min)
Trh-GAL4>UAS-dTrpA1 (n=30)
Trh-GAL4/+ (n=30)
UAS-dTrpA1/+ (n=30)
B
Baseline
Red Light On
Recovery
Cumulative Fraction of Daytime Episodes
C
Cumulative Fraction of Nighttime Episodes
Trh-GAL4>UAS-Chrimson (n=32)
Trh-GAL4/+ (n=32)
UAS-Chrimson/+ (n=31)
D
Activation-Baseline
Red Light On
Recovery-Baseline
Recovery
Δ P(wake)
N.S.
LP
DP
E
Δ P(doze)
Trh-GAL4 (III)>UAS-Chrimson (n=32)
Trh-GAL4 (III)/+ (n=32)
UAS-Chrimson/+ (n=31)

Figure S2. Activation of *Trh-GAL4(III)+* neurons alters the distribution of sleep episodes during both the day and the night, Related to Figure 2. (A-C) Histograms of the cumulative distribution of episode durations during night for thermogenetic activation (A) and during the day (B) and night (C) for optogenetic activation. Baseline day (left), activation day (middle) and recovery day (right) are shown. The experimental genotypes are shown in orange, *UAS/+* and *GAL4/+* controls are in gray. Activation causes a shift from long to short sleep episodes in the experimental genotypes that can be seen as a left shift of the orange curve. (D) Change in P(wake), the probability of transitioning from a sleep state to a wake state is shown for optogenetic activation of *Trh-GAL4(III)+* neurons. (E) Change in P(doze), the probability of transitioning from a wake state to a sleep state is shown for optogenetic activation of *Trh-GAL4(III)+* neurons. Significance is marked for the experimental line when it is different from both controls.

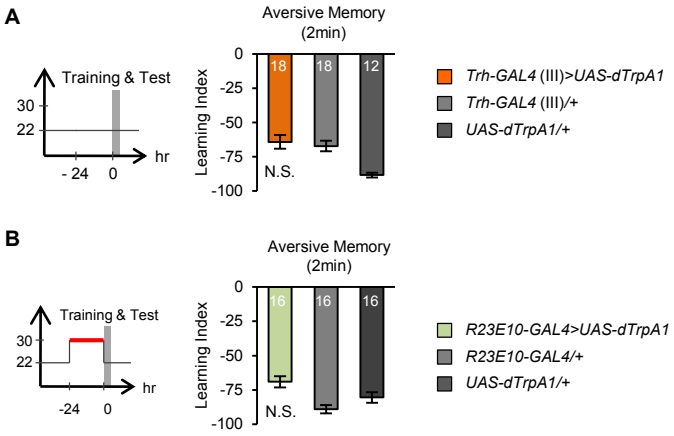

Figure S3. *Trh-GAL4(III)>dTrpA1* flies have normal learning at 22ºC and activation of dorsal fan-shaped body before training does not enhance learning, Related to Figure 3. (A) *Trh(III)>dTrpA1* flies exhibit normal learning ability without activation of dTrpA1 compared to *UAS/+* and *GAL4/+* controls. Left shows schematic of experiment. (B) Activation of the dorsal fan-shaped body with dTrpA1 for 24 h before training does not enhance learning of aversive associations. Left shows schematic of experiment. Red bar indicates activation of dorsal fan-shaped body. Significance is shown for the experimental line when it is different from both genetic controls. The number of independent experiments is indicated on the bars.

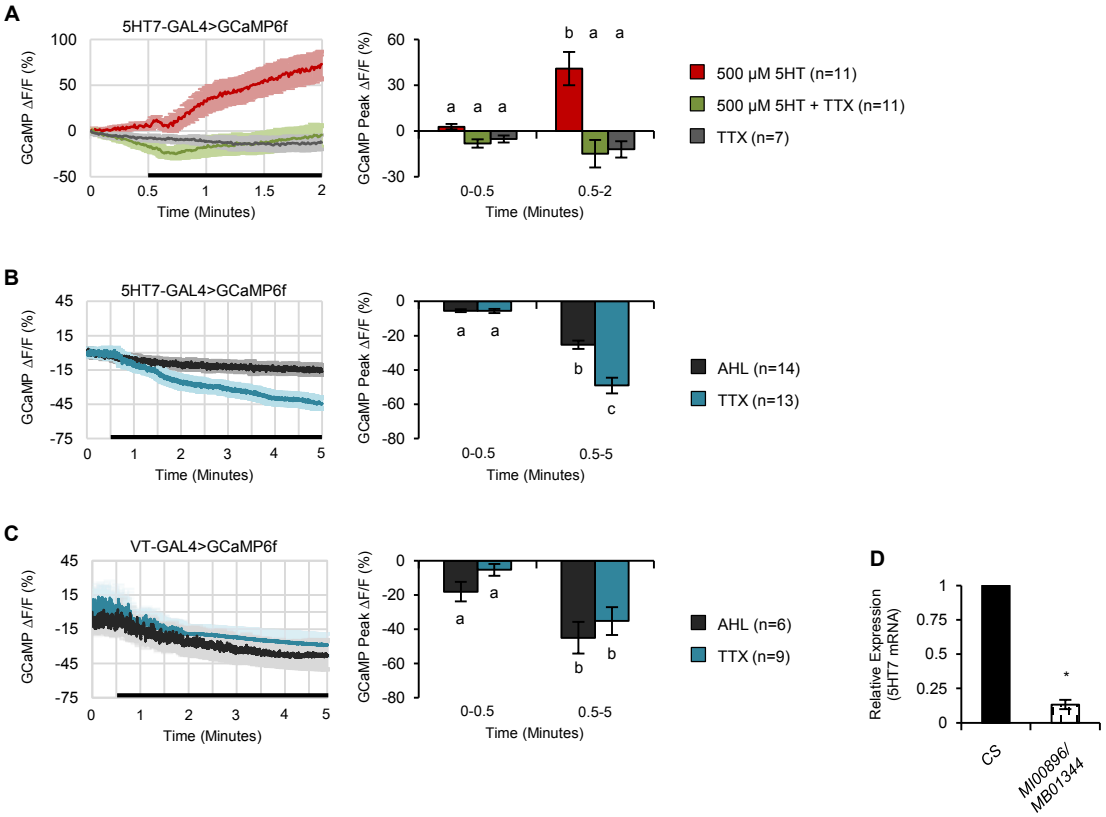

Figure S4. *5HT7-GAL4+* cells in the EB receive excitatory inputs that are active at rest, Related to Figure 4. (A) The response of GCaMP6 in *5HT7-GAL4+* cells in the EB to 500 μM 5HT is shown with and without 1 μM TTX. TTX alone is shown as control. Black bar indicates the period of bath application of the drugs. Right shows quantified data for baseline (the first 30 s of the trace) and drug (last 1.5 min). The ability of 5HT to increase intracellular calcium is completely blocked by TTX, suggesting 5HT is a modulator of some active input to *5HT7-GAL4+* cells. (B) The response of *5HT7-GAL4+* cells in the EB to TTX. Application of AHL vehicle (black) is compared to AHL with 1 μM TTX (blue). TTX causes a statistically significant decrease in calcium, consistent with inhibition of an input pathway. Right shows quantified data. (C) The response of *VT-GAL4+* cells in the EB to TTX. Application of AHL vehicle (black) is compared to AHL with 1 μM TTX (blue). There is no statistically significant change in calcium. Right shows quantified data. Statistically similar groups are marked by the same letter, with different letters indicating significant differences ($p<0.05$) between groups.

(D) *5HT7* mutant transheterozygotes have reduced *5HT7* mRNA. mRNA levels were assessed by RTqPCR (n = 4) and compared to the genetic background *Canton S* wild type control. **** $p<0.0001$.

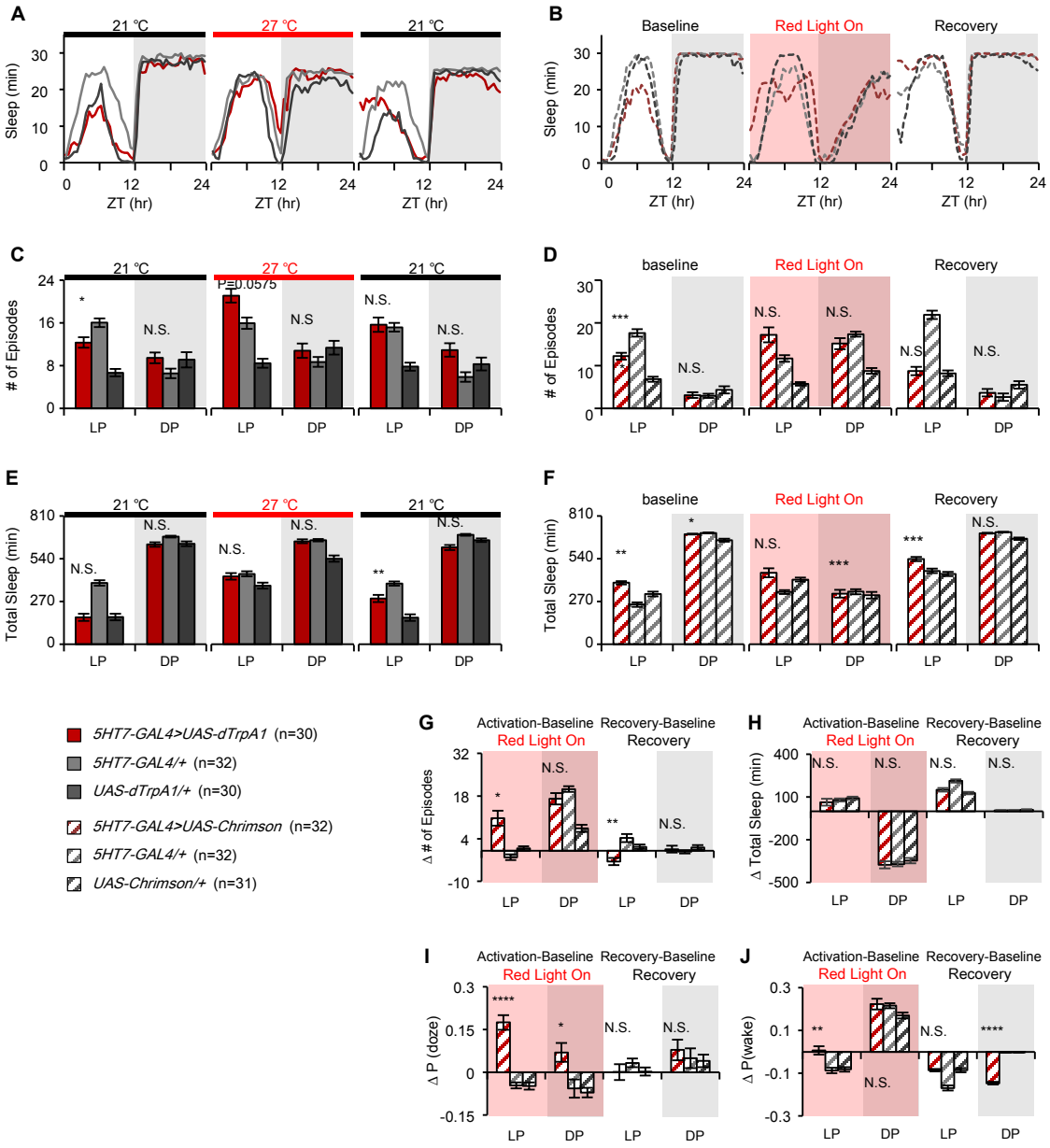
A
21 ℃
27 ℃
21 ℃
Sleep (min)
ZT (hr)
B
Baseline
Red Light On
Recovery
C
P=0.0575
# of Episodes
N.S
LP
DP
D
baseline
E
Total Sleep (min)
F
5HT7-GAL4>UAS-dTrpA1 (n=30)
5HT7-GAL4/+ (n=32)
UAS-dTrpA1/+ (n=30)
5HT7-GAL4>UAS-Chrimson (n=32)
5HT7-GAL4/+ (n=32)
UAS-Chrimson/+ (n=31)
G
Activation-Baseline
Recovery-Baseline
Red Light On
Recovery
Δ # of Episodes
H
Δ Total Sleep (min)
I
Δ P (doze)
J
Δ P(wake)

Figure S5. Complete data set for thermogenetic and optogenetic activation of *5HT7-GAL4*+ neurons, Related to Figure 5. (A, B) Sleep plots. Experimental genotypes are shown in dark red, *UAS/+* and *GAL4/+* controls are shown in gray. Error bars are omitted for clarity. Data are shown for baseline (left), activation (middle) and recovery (right) days. Raw data for the effects of thermogenetic (C) and optogenetic (D) activation on the number of sleep episodes. Raw data for the effects of thermogenetic (E) and optogenetic (F) activation on total sleep. (G) Change in the number of episodes over baseline day with optogenetic activation. (H) Change in total sleep (min) over baseline day with optogenetic activation. (I) Change in P(doze) over baseline day for optogenetic activation. (J) Change in P(wake). Statistical significance for the experimental genotype compared to both controls is shown in the Figure.

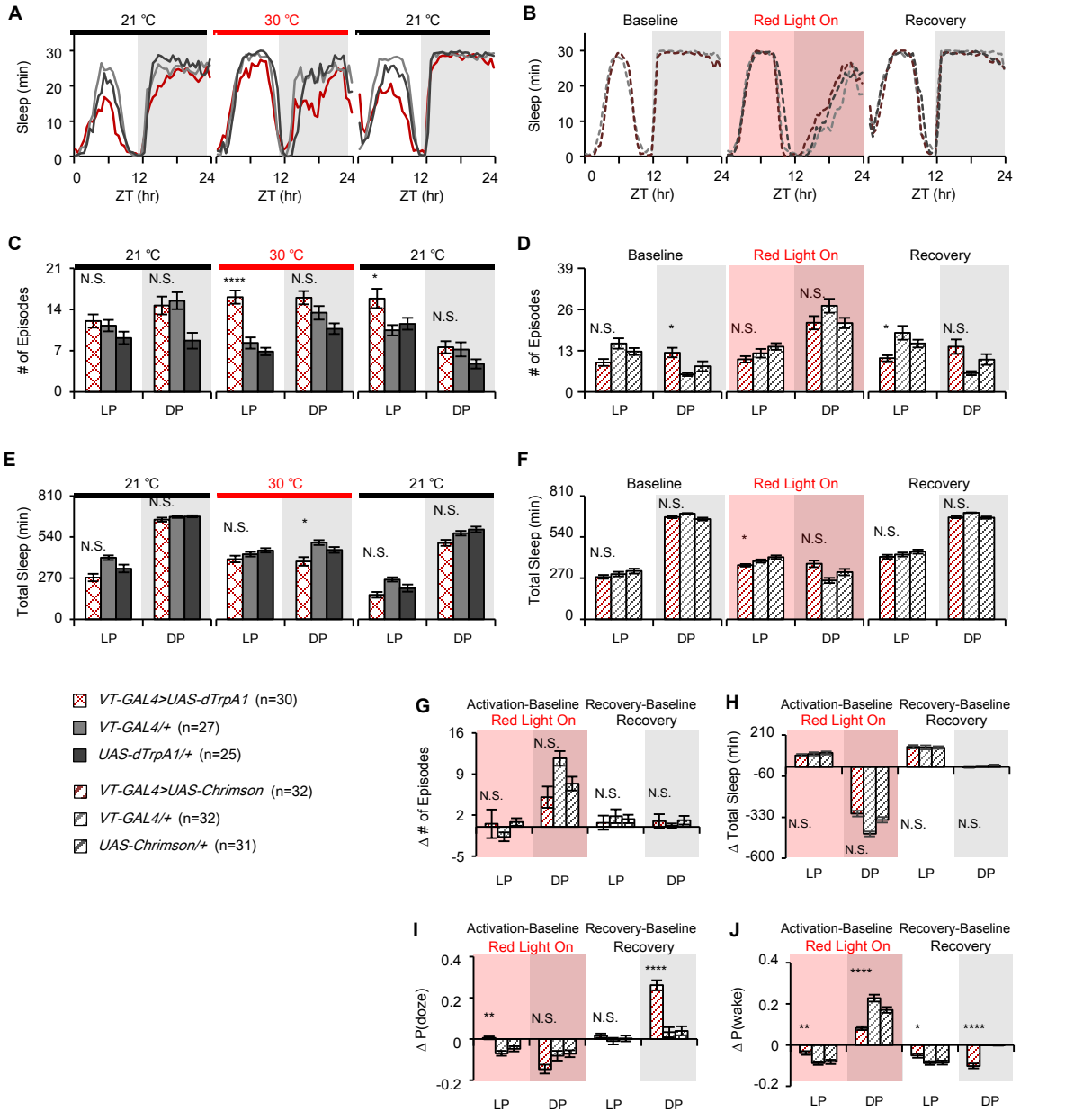
A
21 ℃
30 ℃
21 ℃
Sleep (min)
ZT (hr)
B
Baseline
Red Light On
Recovery
C
# of Episodes
N.S.
****
*
LP
DP
D
E
Total Sleep (min)
F
VT-GAL4>UAS-dTrpA1 (n=30)
VT-GAL4/+ (n=27)
UAS-dTrpA1/+ (n=25)
VT-GAL4>UAS-Chrimson (n=32)
VT-GAL4/+ (n=32)
UAS-Chrimson/+ (n=31)
G
Activation-Baseline
Recovery-Baseline
Red Light On
Recovery
Δ # of Episodes
H
Δ Total Sleep (min)
I
Δ P(doze)
**
J
Δ P(wake)

Figure S6. Complete data set for thermogenetic and optogenetic activation of *VT-GAL4+* neurons, Related to Figure 5. (A,B) Sleep plots. Experimental genotypes are shown in red hatching, *UAS/+* and *GAL4/+* controls are shown in gray. Error bars are omitted for clarity. Data are shown for baseline (left), activation (middle) and recovery (right) days. Raw data for the effects of thermogenetic (C) and optogenetic (D) activation on the number of sleep episodes. Raw data for the effects of thermogenetic (E) and optogenetic (F) activation on total sleep. (G) There is no change in the number of episodes with optogenetic activation over baseline day compared to control genotypes. (H) No change in total sleep (min) with optogenetic activation compared to controls. (I) Change in P(doze). (J) Change in P(wake). Statistical significance for the experimental genotype compared to both controls is shown on the Figure.

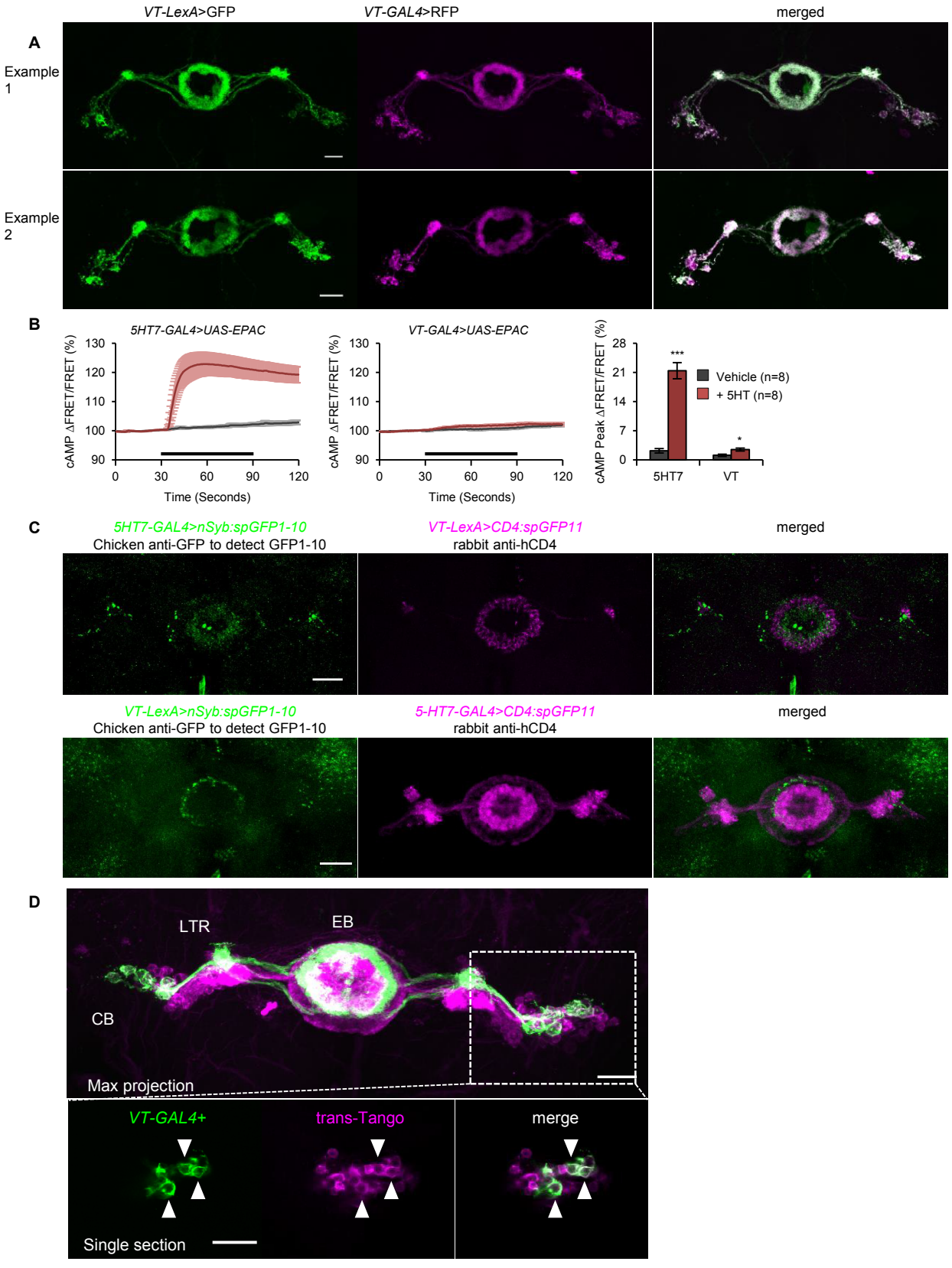
A
VT-LexA>GFP
VT-GAL4>RFP
merged
Example 1
Example 2
B
5HT7-GAL4>UAS-EPAC
VT-GAL4>UAS-EPAC
cAMP ΔFRET/FRET (%)
Time (Seconds)
cAMP Peak ΔFRET/FRET (%)
Vehicle (n=8)
+ 5HT (n=8)
5HT7
VT
C
5HT7-GAL4>nSyb:spGFP1-10
Chicken anti-GFP to detect GFP1-10
VT-LexA>CD4:spGFP11
rabbit anti-hCD4
merged
VT-LexA>nSyb:spGFP1-10
5-HT7-GAL4>CD4:spGFP11
D
LTR
EB
CB
Max projection
VT-GAL4+
trans-Tango
merge
Single section

Figure S7. Verification of different drivers, Related to Figure 4, 6, and 7. (A) Characterization of *VT-LexA* flies. *VT-LexA* was generated using the driver fragment present in *VT-GAL4*. Two samples of the expression pattern of *VT-LexA*. All *VT-LexA*+ neurons overlap with *VT-GAL4*+ neurons, but the GAL4 driver labels 1-2 more neurons than the LexA driver. LexA neurons are visualized with GFP and GAL4 neurons are visualized with RFP. (B) Characterization of 5HT responsiveness of VT neurons. VT neurons have a small cAMP response to 5HT. EPAC signals from *VT-GAL4>EPAC* neurons with application of 100 μM 5HT + 1 μM TTX is shown at right. Quantified data for this experiment is compared to data from *5HT7-GAL4>EPAC* cells at the same dosage of 5HT on the left. Both are statistically significant, but the 5HT7 signal is ca.10-fold larger. Scale bar = 20 μm. (C) Controls for active GRASP. Expression of the spGFP1-10 and spGFP11 fragments was confirmed using 1:1000 chicken anti-GFP, shown in green (Abcam #13970), and 1:500 rabbit anti-hCD4, shown in magenta (Novus #NBP1-86143), respectively. Scale bar = 20 μm. (D) *trans*-Tango shows recurrent connections in 2-3 *VT-GAL4*+ neurons. Expression of the GAL4 is shown in green, *trans*-Tango in magenta and white indicates GAL4+ neurons making synapses on themselves (indicated by arrowheads). Scale bar = 20 μm.

Genotype	Long episode duration cutoff (minutes)	Day 1 - LP Baseline	Day 1 – DP Baseline	Day 2 - LP HEAT (27 ℃)	Day 2 - DP HEAT (27 ℃)	Day 3 – LP Recovery	Day 3 - DP Recovery
TrH(III) > dTrpA	5	1(0)	1(0)	1(0)	1(0)	1(0)	1(0)
	10	1(0)	1(0)	1(0)	1(0)	1(0)	1(0)
	15	1(0)	1(0)	0.97(0.031)	1(0)	0.97(0.031)	1(0)
	30	0.88(0.059)	1(0)	0.63(0.087) *	1(0)	0.91(0.052)	1(0)
	60	0.63(0.087)	0.97(0.031)	0.19(0.07) *	0.94(0.043)	0.81(0.07)	1(0)
	100	0.28(0.081)	0.88(0.059)	0.094(0.052) *	0.56(0.089)	0.56(0.089)	0.97(0.031)
	200	0.13(0.059)	0.63(0.087)	0(0) *	0.13(0.059) *	0.19(0.07)	0.78(0.074)
	300	0.031(0.031)	0.5(0.09)	0(0)	0.031(0.031) *	0.031(0.031)	0.56(0.089)
TrH(III) > +	5	1(0)	1(0)	1(0)	1(0)	1(0)	1(0)
	10	1(0)	1(0)	1(0)	1(0)	1(0)	1(0)
	15	1(0)	1(0)	1(0)	1(0)	0.97(0.031)	1(0)
	30	0.91(0.052)	1(0)	1(0)	1(0)	0.75(0.078)	1(0)
	60	0.69(0.083)	0.97(0.031)	0.94(0.043)	0.94(0.043)	0.47(0.09)	1(0)
	100	0.5(0.09)	0.88(0.059)	0.78(0.074)	0.78(0.074)	0.25(0.078)	0.91(0.052)
	200	0.063(0.043)	0.56(0.089)	0.44(0.089)	0.59(0.088)	0.063(0.043)	0.78(0.074)
	300	0(0)	0.44(0.089)	0.13(0.059)	0.34(0.085)	0(0)	0.63(0.087)
+ > dTrpA	5	1(0)	1(0)	1(0)	1(0)	1(0)	1(0)
	10	1(0)	1(0)	1(0)	1(0)	0.97(0.033)	1(0)
	15	1(0)	1(0)	1(0)	1(0)	0.97(0.033)	1(0)
	30	0.93(0.046)	1(0)	1(0)	1(0)	0.93(0.046)	1(0)
	60	0.87(0.063)	0.93(0.046)	0.93(0.046)	0.9(0.056)	0.7(0.085)	0.93(0.046)
	100	0.57(0.092)	0.87(0.063)	0.87(0.063)	0.73(0.082)	0.53(0.093)	0.9(0.056)
	200	0.23(0.079)	0.4(0.091)	0.43(0.092)	0.53(0.093)	0.17(0.069)	0.57(0.092)
	300	0(0)	0.33(0.088)	0.23(0.079)	0.3(0.085)	0.1(0.056)	0.33(0.088)

Table S1. Analysis of the proportion of individual animals with "long" episodes, Related to Figure 2. Activation of serotonergic neurons decreases episode duration. For each long episode cutoff, the proportion of animals that had at least one episode of that duration was calculated over the course of a three day experiment. * = $p < 0.5$ using ANOVA with Kruskal-Wallis posthoc test.

Genotype	Long episode duration cutoff (minutes)	Day 1 - LP Baseline	Day 1 – DP Baseline	Day 2 - LP HEAT (27 ℃)	Day 2 - DP HEAT (27 ℃)	Day 3 – LP Recovery	Day 3 - DP Recovery
TrH(III) > dTrpA	5	1(0)	1(0)	1(0)	1(0)	1(0)	1(0)
	10	0.8(0.032)	0.97(0.0076)	0.69(0.036) *	0.91(0.013)	0.85(0.024)	0.98(0.0051)
	15	0.72(0.04)	0.94(0.015)	0.49(0.04) *	0.84(0.022)	0.76(0.032)	0.95(0.0091)
	30	0.59(0.053)	0.86(0.03)	0.2(0.04) *	0.64(0.033)	0.6(0.045)	0.9(0.017)
	60	0.42(0.064)	0.7(0.05)	0.069(0.03) *	0.39(0.04) *	0.46(0.05)	0.78(0.035)
	100	0.18(0.058)	0.6(0.057)	0.022(0.013) *	0.21(0.041) *	0.31(0.054)	0.64(0.045)
	200	0.11(0.055)	0.4(0.063)	0(0) *	0.064(0.032) *	0.11(0.044)	0.49(0.058)
	300	0.029(0.029)	0.33(0.064)	0(0)	0.018(0.018) *	0.019(0.019)	0.4(0.069)
TrH(III) > +	5	1(0)	1(0)	1(0)	1(0)	1(0)	1(0)
	10	0.81(0.023)	0.97(0.0063)	0.9(0.013)	0.94(0.016)	0.78(0.029)	0.98(0.0071)
	15	0.71(0.035)	0.93(0.014)	0.85(0.017)	0.89(0.024)	0.63(0.046)	0.96(0.013)
	30	0.51(0.047)	0.82(0.036)	0.71(0.028)	0.78(0.038)	0.43(0.058)	0.89(0.029)
	60	0.38(0.054)	0.68(0.053)	0.61(0.043)	0.65(0.052)	0.27(0.056)	0.77(0.046)
	100	0.3(0.058)	0.62(0.06)	0.49(0.057)	0.52(0.066)	0.14(0.047)	0.68(0.059)
	200	0.054(0.038)	0.43(0.075)	0.34(0.07)	0.39(0.068)	0.048(0.033)	0.59(0.067)
	300	0(0)	0.33(0.072)	0.11(0.051)	0.25(0.066)	0(0)	0.47(0.071)
+ > dTrpA	5	1(0)	1(0)	1(0)	1(0)	1(0)	1(0)
	10	0.88(0.022)	0.96(0.0088)	0.94(0.0094)	0.93(0.014)	0.85(0.033)	0.96(0.0092)
	15	0.84(0.034)	0.93(0.016)	0.9(0.014)	0.88(0.021)	0.78(0.037)	0.93(0.015)
	30	0.7(0.05)	0.82(0.034)	0.75(0.034)	0.75(0.039)	0.63(0.05)	0.81(0.036)
	60	0.6(0.053)	0.61(0.053)	0.62(0.052)	0.57(0.055)	0.46(0.064)	0.66(0.047)
	100	0.37(0.064)	0.5(0.058)	0.54(0.059)	0.45(0.061)	0.38(0.07)	0.51(0.05)
	200	0.57(0.092)	0.87(0.063)	0.87(0.063)	0.73(0.082)	0.53(0.093)	0.9(0.056)
	300	0(0)	0.23(0.062)	0.2(0.07)	0.21(0.059)	0.079(0.045)	0.22(0.062)

Table S2. Analysis of the proportion of total sleep accounted for by "long" episodes, Related to Figure 2. For each episode duration cutoff, the proportion of total sleep that was made of up episodes of that length or greater was calculated. LP is light period, DP is dark period. Value present is mean. SEM is in parentheses. * = $p < 0.5$ using ANOVA with Kruskal-Wallis posthoc test.