SUPPLEMENTAL INFORMATION

Figure S1

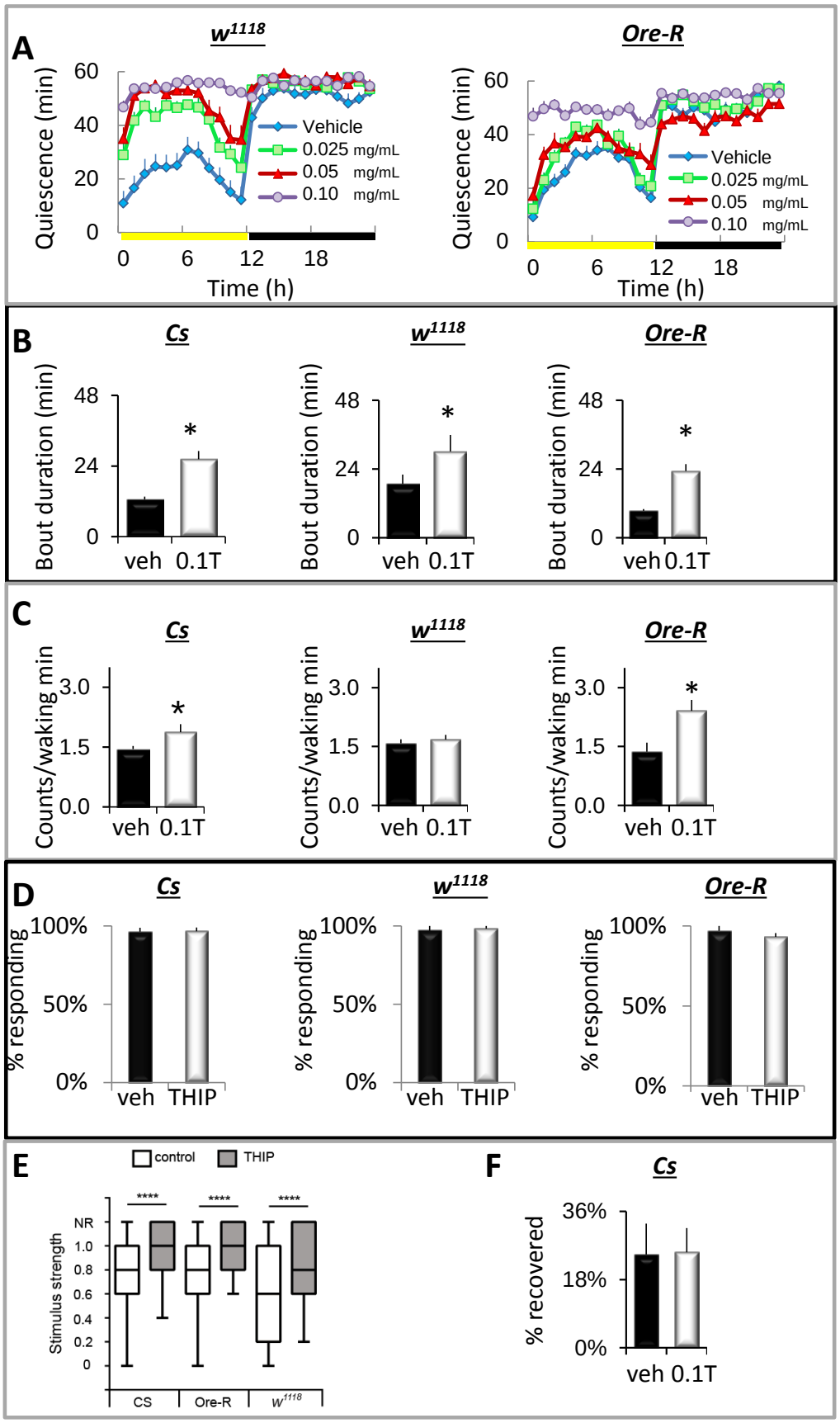

Figure S1. **Related to Figure 1. THIP increases sleep in common background strains. (A)** THIP increases quiescence (min/h) in a dose-dependent manner in w^{1118} and *Ore-R* flies. Data are presented as sleep in minutes/hour. Repeated measures ANOVAs reveals a significant Dose (4) X Hour (24) interaction for each genotype (w^{1118}: $F_{(69,1196)}$=7.62, p= 9.99^{E-16} ,n=14-16/group and *Ore-R* $F_{(69,1311)}$=3.08, p= 5.11^{E-15},n=14-16/group. **(B)** Flies maintained on 0.1mg/mL (0.1T) THIP (white bars) significantly increased sleep bout duration during the day compared to vehicle-fed controls (veh, black bars), (*Cs* ttest P=1.46^{E-11}; w^{1118} ttest p=0.04; and *Ore-R* ttest p= 2.16^{E-06}. **(C)** The intensity of waking locomotor activity during the day is increased during THIP treatment in *Cs* and *Ore-R* flies (*Cs* ttest, P= 0.01; *Ore-R* ttest p=0.006) and is unchanged in w^{1118} flies (ttest, p=0.20); data are presented as counts per waking minute (c/w min). **(D)** THIP induced sleep is rapidly reversible. (A-C) *Cs*, w^{1118} and *Ore-R* flies were maintained in the dark on either vehicle or THIP and exposed to a single 10 minute light pulse to determine if they could rapidly wake up. Only flies that had been sleeping were evaluated. The % of flies that responded during the light pulse was tabulated for each genotype and drug condition; 4-6 replicates were run for each genotype and condition. A 3(Genotype) X 2(Vehicle, THIP) ANOVA did not find a main effect for Drug, a main effect for genotype nor a genotype X Drug interaction: $F_{[1,24]}$ = 0.1; p=0.75; $F_{[2,24]}$ = 0.45; p=0.641 and $F_{[1,24]}$ = 0.1; p=0.75; $F_{[2,24]}$ = 0.46; p=0.63 **(E)** Arousal thresholds for *Cs*, *Ore-R* and w^{1118} strains with or without THIP (0.1mg/ml), measured by responses to vibrational stimuli of different strengths. Boxplots show the median (center line), interquartile range (25th/75th percentiles) and whiskers (95th/5th percentiles) for the arousal threshold, the weakest stimulus to which flies responded. Stimulus strength is indicated as a proportion of the maximum vibrational strength, 1.0 = 1.2g), n>720 tests, 30 flies; ****p<0.0001, Wilcoxon rank-sum test. **(F)** Both vehicle-fed and THIP-fed *Cs* flies compensate for lost sleep as indicated by exhibiting a wild-type sleep rebound during 48 h of recovery following 12 h of sleep deprivation, ttest, P=0.93; n=27/group. Error bars, s.e.m.;*P<0.05.

Figure S2

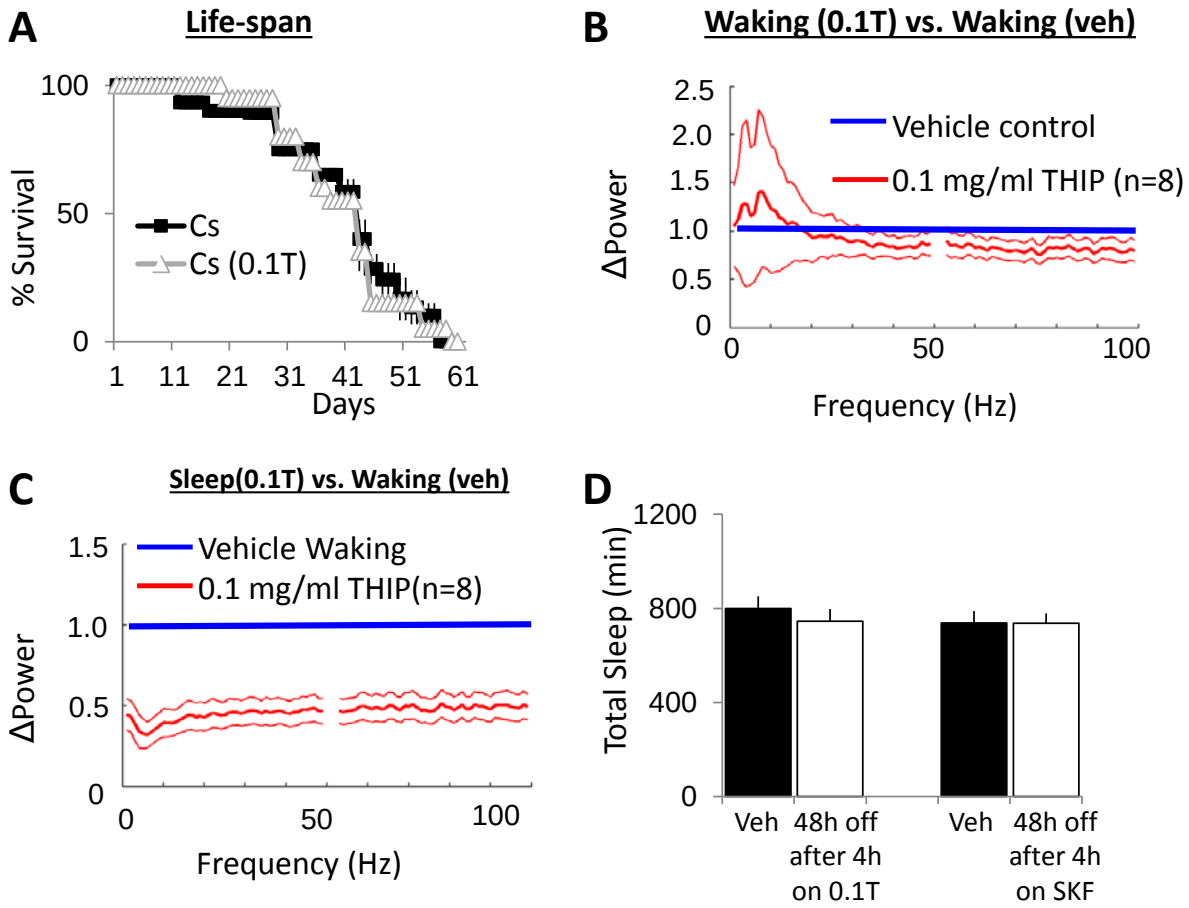

Figure S2. Related to Figure 1.THIP induced sleep does not alter physiology. (A) Three-day-old *Cs* flies were maintained on food or 0.1mg/mL (0.1T)THIP and lifespan was monitored. No changes in lifespan were observed between controls and 0.1T (n=3 groups of 10/condition). (**B)** Quantification of mean spectral analysis plotting the difference in power (ΔPower) between waking while on 0.1mg/mL THIP (red lines ± s.e.m.) compared to waking while on Vehicle (Blue) (n= 8 flies). **(C)** THIP-induced sleep (red lines ± s.e.m.) is associated with a uniform decrease in spectral power across all frequencies compared with waking (Blue). All THIP experiments were normalized to vehicle controls performed in the same animal prior to drug feeding. **(D)**Total sleep in *Cs* flies 48 h after exposure to 4 h of either THIP or SKF (white) compared to vehicle fed controls (Black). Error bars, s.e.m.;*P<0.05.

Figure S3

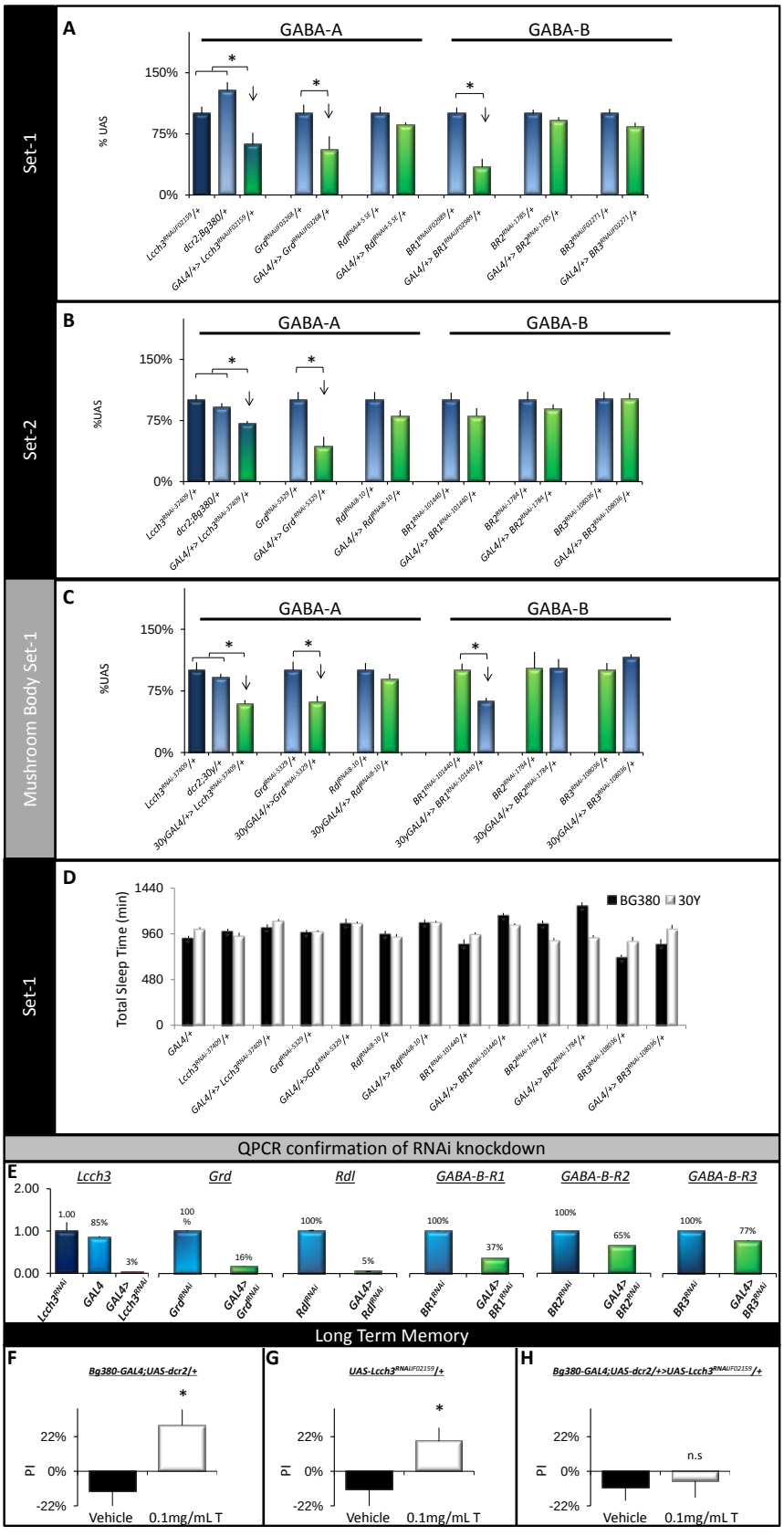
Set-1
A
GABA-A
GABA-B
% UAS
150%
75%
0%
Set-2
B
%UAS
Mushroom Body Set-1
C
Set-1
D
Total Sleep Time (min)
1440
960
480
0
BG380
30Y
QPCR confirmation of RNAi knockdown
E
Lcch3
Grd
Rdl
GABA-B-R1
GABA-B-R2
GABA-B-R3
2.00
1.00
0.00
Long Term Memory
F
Bg380-GAL4;UAS-dcr2/+
G
UAS-Lcch3RNAiJF02159/+
H
Bg380-GAL4;UAS-dcr2/+>UAS-Lcch3RNAiJF02159/+
PI
22%
0%
-22%
Vehicle
0.1mg/mL T
n.s

Figure S3. Related to Figure 1. THIP acts through the *Lcch3* and *Grd* GABA-A receptors. (A, B) Two independent UAS-RNAi lines for each of the 6 *Drosophila* GABA receptors were expressed using *BG380-GAL4;dcr2*. Only *Lcch3* and *Grd* RNAi lines consistently attenuated their response to THIP compared to their respective parental controls. Sleep in response to THIP is expressed as % of the corresponding THIP-fed UAS control. One way ANOVA for genotype, *<0.05, modified Bonferroni test, n=11-15 flies/group. **(C)** UAS-RNAi lines from Set-1 for each of the 6 *Drosophila* GABA receptors were also expressed using *Dr2;30y*-GAL4since this driver has been shown to be involved in wake-regulating circuitry. Sleep in response to THIP is expressed as % of the corresponding THIP-fed UAS control; *<0.05, modified Bonferroni test, n=12-16 flies/group. **(D)** Total sleep in RNAi lines for each of the 6 *Drosophila* GABA receptors when expressed using *BG380-GAL4;Dcr2* (Black)or *Dcr2;30Y* (white). All values fall within the rage commonly observed for wild-type strains. **(E)** Relative transcript levels as assessed by QPCR from RNAi set-1 are expressed as % of the corresponding UAS controls. **(F)** Male *Bg380-GAL4; dcr2/+* parental controls exposed to 3 h of training and maintained on vehicle for 4 h do not display long-term memory (LTM) when tested 48 h later. However, increasing sleep by placing flies on THIP for 4 h immediately following training resulted in an LTM as measured by courtship suppression, p=0.01 Kruskal-Wallis Test, n=15-16/group. **(G)** No courtship suppression is seen in $Lcch3^{RNAiJF02159}/+$ parental controls maintained on vehicle following training but is readily observed in siblings whose sleep has been increased by the administration of THIP for 4 h post-training; p=0.03 Kruskal-Wallis Test, n=14-16/group. **(H)** THIP does not support LTM following training in *BG380-GAL4; dcr2/+>UAS-*$Lcch3^{RNAiJF02159}/+$ flies; p=0.85 Kruskal-Wallis Test, n=14-16/group. Error bars, s.e.m.;*P<0.05.

Figure S4

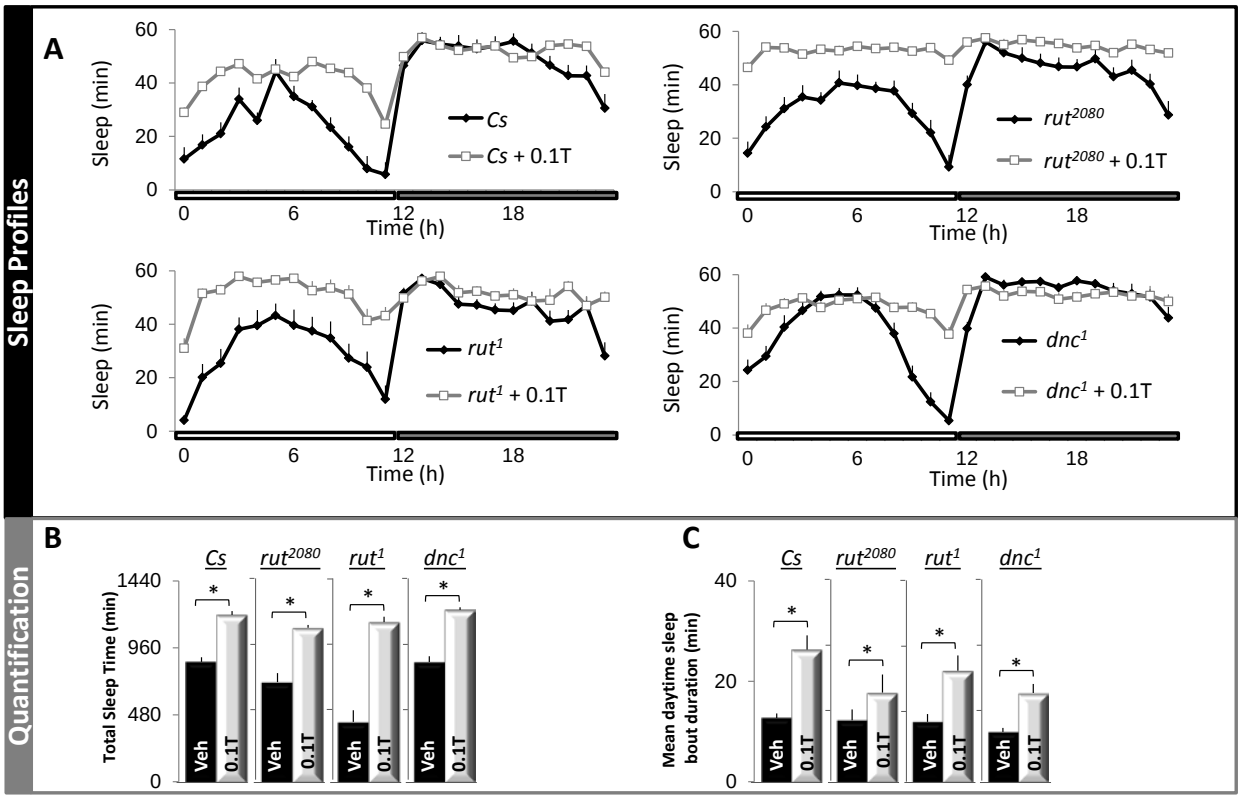

Figure S4. Related to Figure 2. THIP increases sleep in *rut* and *dnc* mutants. (A) Sleep in minutes/h for *Cs*, *rut*^{2080}, *rut*^{1} and *dnc*^{1} mutants maintained on vehicle or fed 0.1mg/mL THIP (0.1T). Data are presented as mean ± s.e.m., n=16/genotype. **(B)** Quantification of data presented in panel A; *p<0.05 ttest. **(C)** THIP increases sleep consolidation during the day as defined by an increase in the mean sleep bout duration, *p<0.05 ttest. Error bars, s.e.m.;*P<0.05.

Figure S5

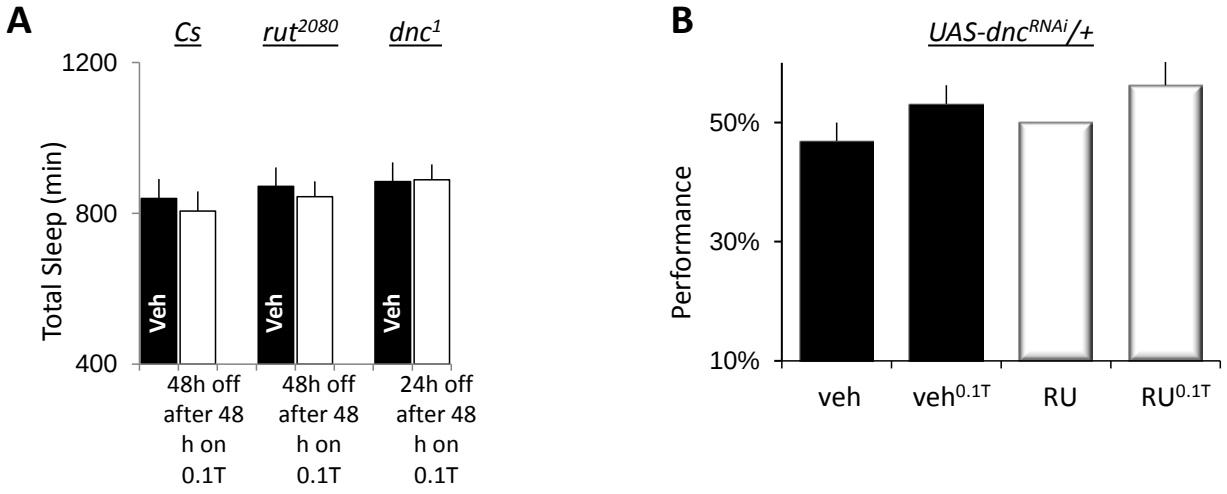

Figure S5. Related to Figure 3. Sleep returns to baseline after removal from THIP. (A) Total sleep in *Cs*, *rut*^{2080} and *dnc*^{1} flies after exposure to 48 h of THIP (white) compared to vehicle fed controls (Black). Sleep time is shown 48 h and 24 h after being removed from THIP for *rut*^{2080} and *dnc*^{1} respectively. **(B)**Short-term memory in *UAS-dncRNAi/+* parental controls is unaffected by either RU or 0.1mg/mL THIP administration. A 2 (veh, RU) X2(Veh, TIP) revealed no interaction ANOVA $F_{[3,28]} = 1.79$; p=0.17. Error bars, s.e.m.;*P<0.05.

Table S1

	TCT (Time to complete test)			PI (Phototaxis index)				QSI (Quinine sensitivity index)			
	mean	±	s.e.m.	mean	±	s.e.m.	n	mean	±	s.e.m	n
Figure 2A											
Cs:											
Baseline	7.32	±	0.32	84%	±	5%	6	212	±	42	6
THIP	7.38	±	0.35	83%	±	3%		279	±	8	
W^{1118}:											
Baseline	7.28	±	0.18	68%	±	7%	6	183	±	20	5
THIP	7.85	±	0.73	66%	±	5%		177	±	18	
Ore R:											
Baseline	8.12	±	0.23	86%	±	5%	6	238	±	12	6
THIP	8.51	±	0.37	85%	±	5%		222	±	28	
ry^{506}:											
Baseline	13.00	±	0.18	87%	±	4%	6	252	±	9	6
THIP	11.25*	±	0.25	95%	±	5%		255	±	9	
Berlin:											
Baseline	10.75	±	0.37	79%	±	1%	6	247	±	14	6
THIP	11.50	±	0.19	84%	±	3%		240	±	14	
Figure 2B											
rut^{2080}:											
Baseline	12.33	±	0.21	65%	±	9%	6	225	±	20	6
THIP	12.36	±	0.57	82%	±	6%		196	±	34	
THIP + 12 h SD	11.00	±	0.42	78%	±	6%		186	±	15	
Figure 2C											
rut^{1}:											
Baseline	12.50	±	0.32	82%	±	3%	6	270	±	8	6
THIP	13.25	±	0.25	90%	±	2%		263	±	10	
THIP + 12 h SD	13.50	±	0.18	80%	±	2%		271	±	8	
Figure 2G-I											
DaGsw-GAL4/+											
Veh	9.60	±	0.18	85%	±	2%	6	239	±	26	6
Veh + THIP	9.87	±	0.35	93%	±	2%		283	±	15	
RU	9.62	±	0.26	83%	±	2%		270	±	8	
RU +THIP	10.87	±	0.35	83%	±	2%		263	±	25	
rut^{RNAi}/+											
Veh	8.87	±	0.35	75%	±	2%	6	201	±	11	6
Veh + THIP	9.37	±	0.26	78%	±	2%		258	±	23	
RU	8.87	±	0.29	75%	±	2%		180	±	41	
RU +THIP	9.13	±	0.13	77%	±	2%		184	±	40	
DaGsw/+>rut^{RNAi}/+											
Veh	9.13	±	0.35	83%	±	2%	6	228	±	43	6
Veh + THIP	9.50	±	0.19	83%	±	3%		277	±	22	
RU	9.38	±	0.18	83%	±	2%		240	±	38	
RU +THIP	10.00	±	0.27	83%	±	2%		265	±	33	
Figure 2L											
***rut^{2080}* FB activation:**											
rut^{2080};104y/+	8.62	±	0.32	90%	±	4%	6	260	±	9	6
rut^{2080}; NaChBac/+	8.25	±	0.16	100%	±	0%		251	±	12	
rut^{2080};104y/+>NaChBac/+	12.50*	±	0.61	90%	±	6%		263	±	12	
rut^{2080};;c5/+	10.37	±	0.29	80%	±	5%		256	±	13	
rut^{2080};;c5/+>NaChBac/+	11.12*	±	0.76	98%	±	1%*		266	±	14	
Figure 2M											

***rut*^{2080} FB Time Course (24 h)**												
rut^{2080}*;104y/+* 25°C	12.37	±	0.18	83%	±	3%	6	269	±	8	6
rut^{2080}*;UAS-TrpA1/+* 25°C	12.50	±	0.18	80%	±	3%		249	±	23	
rut^{2080}*;104y/+>TrpA1/+* 25°C	12.62	±	0.26	78%	±	3%		266	±	7	
rut^{2080}*;104y/+* 31°C	12.50	±	0.18	81%	±	5%		252	±	11	
rut^{2080}*;UAS-TrpA1/+* 31°C	11.75	±	0.16	80%	±	5%		265	±	7	
rut^{2080}*;104y/+>TrpA1/+* 31°C	12.25	±	0.16	80%	±	4%		215	±	21	
Figure 2N											
w(isoCJ1)											
20°C	10.50	±	0.46	75%	±	3%	6	262	±	32	5
30°C	11.22	±	0.49	68%	±	5%		267	±	33	
Figure 2O											
dFabp/+											
20°C	11.50	±	0.57	75%	±	3%	6	202	±	33	6
30°C	11.60	±	0.50	69%	±	4%		265	±	27	
Figure 2P											
rut*^{2080}*;;dFabp/+											
20°C	10.58	±	0.47	73%	±	3%	6	182	±	20	6
30°C	11.40	±	0.54	73%	±	4%		252	±	36	
30°C+12h SD	15.12*	±	0.72	73%	±	3%		231	±	25	

Table S1. Related to Figure 2. Time to Complete Test (TCT), Phototaxis Index (PI) and the Quinine Sensitivity Index (QSI) for all genotypes tested for short-term memory (STM) in Figure2. The TCT represents the observed time to complete 16 trials during training; thus, the sample size is the same as the corresponding figure in the text. The phototaxis index (PI) is calculated as the average proportion of visits to the light alley of the T-maze during 10 trials in the absence of quinine. The Quinine Sensitivity Index (QSI) is determined by calculating the time that the fly spent on the dry side of the tube when the other side had been wetted with quinine during a 5 min period. The final PI and QSI is the average of the scores obtained for 5-6 flies ± s.e.m.. All flies display TCT, PI and QSI scores well within ranges that permit normal learning[S1, S2]. Red letters indicate significant difference from both parental controls while green letters indicate a difference from one parental control. The corresponding t-tests and ANOVAs for the results in Table S1 can be found in Table S4.

Table S2

	TCT (Time to complete test)			PI (Phototaxis index)				QSI (Quinine sensitivity index)			
	mean	±	s.e.m.	mean	±	s.e.m.	n	mean	±	s.e.m	n
Figure 3A											
***dnc^{1}*:**											
Baseline	10.50	±	0.32	73%	±	4%	6	171	±	22	6
THIP	11.12	±	0.71	63%	±	7%		181	±	27	
THIP + 12 h SD	8.87*	±	0.22	76%	±	2%		192	±	26	
Figure 3E-S5B											
***dnc^{RNAi}*/+**											
Veh	10.37	±	0.18	87%	±	2%	6	260	±	9	6
Veh + THIP	11.25	±	0.31	85%	±	2%		250	±	17	
RU	9.50	±	0.18	88%	±	2%		229	±	11	
RU +THIP	11.00	±	0.32	87%	±	2%		224	±	22	
DaGsw/+>dnc^{RNAi}/+											
Veh	10.62	±	0.37	80%	±	0%	6	258	±	34	6
Veh + THIP	11.25	±	0.25	88%	±	2%		230	±	43	
RU	11.20	±	0.32	83%	±	2%		270	±	20	
RU +THIP	11.25	±	0.36	90%	±	0%		240	±	16	
Figure 3F											
***dnc^{1}* FB activation:**											
dnc^{1};104y	9.87	±	0.76	90%	±	4%	6	241	±	8	6
dnc^{1}; NaChBac/+	12.25	±	0.31	95%	±	3%		260	±	4	
dnc^{1};104y/+>NaChBac/+	11.62*	±	0.37	95%	±	2%		262	±	17	
dnc^{1};;c5	10.75	±	0.49	96%	±	2%		222	±	12	
dnc^{1};;c5/+>NaChBac/+	11.25*	±	0.25	90%	±	4%		224	±	30	
Figure 3G											
***dnc^{1}* FB Time Course (24 h)**											
dnc^{1};104y/+ 25°C	12.50	±	0.18	86%	±	3%	6	250	±	9	6
dnc^{1};UAS-TrpA1/+ 25°C	12.12	±	0.29	83%	±	3%		246	±	15	
dnc^{1};104y/+>TrpA1/+ 25°C	12.62	±	0.18	80%	±	3%		206	±	19	
dnc^{1};104y/+ 31°C	12.37	±	0.18	71%	±	4%*		207	±	16	
dnc^{1};UAS-TrpA1/+ 31°C	12.50	±	0.18	85%	±	3%		266	±	7	
dnc^{1};104y/+>TrpA1/+ 31°C	12.25	±	0.25	90%	±	4%		241	±	23	
Figure 3I											
dnc^{1};;dFabp/+											
20°C	11.00	±	0.52	77%	±	3%	6	204	±	26	6
30°C	11.54	±	0.39	75%	±	4%		259	±	24	
30°C + 12h SD	13.63*	±	0.63	73%	±	3%		145	±	33	

Table S2. Related to Figure 3. Time to Complete Test (TCT), Phototaxis Index (PI) and the Quinine Sensitivity Index (QSI) for all genotypes tested for short-term memory (STM) in Figure3. The corresponding t-tests and ANOVAs for the results in Table S2 can be found in Table S4.

Table S3

	TCT (Time to complete test)			PI (Phototaxis index)				QSI (Quinine sensitivity index)			
	mean	±	s.e.m.	mean	±	s.e.m.	n	mean	±	s.e.m	n
Figure 4C											
rut^{2080};*104y*/+ Veh	14.50	±	0.57	78%	±	4%	6	173	±	15	5
rut^{2080};*104y*/+ THIP	14.50	±	0.63	77%	±	2%		139	±	29	
rut^{2080};*UAS-kir2.1*/+ Veh	14.13	±	0.44	75%	±	2%		158	±	12	
rut^{2080};*UAS-kir2.1*/+ THIP	11.43	±	0.69	75%	±	4%		212	±	43	
rut^{2080};*104y/+>UAS-kir2.1* Veh	12.63	±	0.89	78%	±	2%		191	±	29	
rut^{2080};*104y/+>UAS-kir2.1* THIP	12.10	±	0.75	78%	±	3%		174	±	11	
Figure 4F											
aru$^{8.128}$/+	8.63	±	0.26	95%	±	3%	6	261	±	12	6
rut^{2080};*aru*$^{8.128}$/+	9.13	±	0.23	90%	±	3%*		260	±	9	
Figure 4G											
DaGsw/+>aru^{RNAi}/+											
Veh	10.56	±	0.47	71%	±	4%	6	292	±	3	6
RU	11.75	±	0.25	72%	±	3%		278	±	12	
Figure 4H											
dnc^{1};DaGsw/+>aru^{RNAi}/+											
Veh	10.82	±	0.26	70%	±	7%	6	251	±	23	5
RU	11.40	±	0.27	77%	±	2%		262	±	19	
Figure 4I											
rut^{2080};DaGsw/+>aru^{RNAi}/+											
Veh	13.25	±	0.22	77%	±	6%	6	207	±	53	5
RU	12.40*	±	0.16	83%	±	2%		149	±	31	

Table S3. Related to Figure 4. Time to Complete Test (TCT), Phototaxis Index (PI) and the Quinine Sensitivity Index (QSI) for all genotypes tested for short-term memory (STM) in Figure4. The corresponding t-tests and ANOVAs for the results in Table S3 can be found in Table S4.

Table S4

Genotype	TCT	PI	QSI
Cs	p=0.17	p=0.91	p=0.19
w^{1118}	p=0.46	p= 0.13	p=0.83
Ore-R	p=0.36	p=0.89	p=0.63
ry^{506}	p=6.73E-05	p=0.23	p=0.78
Berlin	p=0.09	p=0.12	p=0.73
rut^{2080}	$F_{[2,22]}$ = 2.29; p=0.13	$F_{[2,14]}$ = 1.4; p=0.26	$F_{[2,13]}$ = 0.69; p=0.51
rut^{1}	$F_{[2,21]}$ = 3.95; p=0.03	$F_{[2,16]}$ = 2.71; p=0.09	$F_{[2,15]}$ = 0.17; p=0.84
DaGs-GAL4/+	$F_{[1,28]}$ = 2.87; p=0.10	$F_{[1,20]}$ = 3.78; p=0.06	$F_{[1,19]}$ = 1.66; p=0.21
rut^{RNAi}/+	$F_{[1,28]}$ = 0.21; p=0.64	$F_{[1,20]}$ = 0.16; p=0.69	$F_{[1,20]}$ = .72; p=0.41
DaGs/+>rut^{RNAi}/+	$F_{[1,28]}$ = 0.24; p=0.63	$F_{[1,20]}$ = 0.00; p=1.00	$F_{[1,20]}$ = 0.11; p=0.73
rut^{2080}*;104y/+>NaChBac/+*	$F_{[2,19]}$ = 36.53; p=3.06E-07	$F_{[2,15]}$ = 1.875; p=0.18	$F_{[2,15]}$ = 0.15; p=0.85
rut^{2080}*;c5/+>NaChBac/+*	$F_{[2,21]}$ = 6.41; p=0.006	$F_{[2,15]}$ = 10.23; p=0.001	$F_{[2,15]}$ = 0.29; p=0.74
rut^{2080}*;104y/+>TrpA1/+*	$F_{[2,42]}$=1.17; p=0.31	$F_{[2,30]}$=0.086; p=0.91	$F_{[2,30]}$=2.62; p=0.089
w(isoCJ1)	$F_{[1,15]}$=1.12; p=0.30	$F_{[1,10]}$=1.29; p=0.28	$F_{[1,8]}$=0.009; p=0.92
dFabp/+	$F_{[1,16]}$=0.01; p=0.89	$F_{[1,11]}$=1.19; p=0.30	$F_{[1,10]}$=2.21; p=0.17
rut^{2080}*;;dFabp/+*	$F_{[2,27]}$ = 16.94; p=1.71E-05	$F_{[2,15]}$ = 0; p=1	$F_{[2,15]}$ = 1.73; p=0.21
dnc^{1}	$F_{[2,21]}$ = 6.00; p=0.008	$F_{[2,22]}$ = 1.39; p=0.26	$F_{[2,13]}$ = 0.12; p=0.88
dnc^{RNAi}/+	$F_{[1,28]}$ = 1.43; p=0.24	$F_{[1,20]}$ = 0.00; p=1.00	$F_{[1,20]}$ = 0.01; p=0.89
DaGs/+>dnc^{RNAi} /+	$F_{[1,30]}$ = 0.73; p=0.39	$F_{[1,20]}$ = 0.38; p=0.54	$F_{[1,20]}$ = 0.01; p=0.97
dnc^{1}*;104Y/Nachbac4*	$F_{[2,21]}$ = 5.5; p=0.01	$F_{[2,15]}$ = 0.83; p=0.45	$F_{[2,15]}$ = 1.09; p=0.36
dnc^{1}*;c5/+>NaChBac/+*	$F_{[2,21]}$ = 4.3; p=0.02	$F_{[2,15]}$ = 1; p=0.39	$F_{[2,15]}$ = 1.29; p=0.30
dnc^{1}*;104y/+>TrpA1/+*	$F_{[2,45]}$=0.81;p=0.44	$F_{[2,30]}$=5.41;p=0.01	$F_{[2,30]}$=3.24;p=0.055
dnc^{1}*;;dFabp/+*	$F_{[2,28]}$=6.80;p=0.003	$F_{[2,15]}$ = 0.20; p=0.81	$F_{[2,15]}$ = 3.62; p=0.06
rut^{2080}*;104y/+>kir2.1/+*	$F_{[2,43]}$=2.04;p=0.14	$F_{[2,30]}$=0.05;p=0.95	$F_{[2,24]}$=1.64;p=0.22
rut^{2080}*;*$aru^{8.128}$*/+*	$F_{[2,22]}$=1.28;p=0.30	$F_{[2,15]}$=7.5;p=0.005	$F_{[2,14]}$=1.96;p=0.18
DaGs/+>aru^{RNAi}/+	$F_{[1,15]}$=4.59;p=0.05	$F_{[1,11]}$=0.002;p=0.96	$F_{[1,10]}$=1.39;p=0.26
dnc^{1}*;DaGs/+>*aru^{RNAi}/+	$F_{[1,19]}$=2.40;p=0.14	$F_{[1,10]}$=0.77;p=0.40	$F_{[1,8]}$=0.14;p=0.71
rut^{2080}*;DaGs/+>*aru^{RNAi}/+	$F_{[1,20]}$=9.11;p=0.007	$F_{[1,10]}$=1.05;p=0.33	$F_{[1,8]}$=0.88;p=0.37

Table S4. Statistics for Time to Complete (TCT), Phototaxis Index (PI) and Quinine Sensitivity Index (QSI). t-tests were conducted on common background strains (*Cs*, w^{1118}, *Ore-R*, ry^{506} and *Berlin*). ANOVAS were conducted for all other experiments followed by modified Bonferroni comparisons.

SUPPLEMENTAL EXPERIMENTAL PROCEDURES

Flies

Flies were cultured at 25˚C with 50-60% relative humidity and kept on a diet of yeast, dark corn syrup and agar under a 12-hour light:12-hour dark cycle. *104y-GAL4* and *C5-GAL4* flies were obtained from M. Heisenberg (Rudolf Virchow Center). *BG380-GAL4; UAS-dcr2* were obtained from A. DiAntonio (Washington University School of Medicine in St. Louis). *UAS-NaChBac* flies were obtained from A. Sehgal (University of Pennsylvania). *w(isoCJ1)* and *dFabp* flies were obtained from JC Yin (University of Madison Wisconsin). $aru^{8.128}$ and aru^{RNAi} flies were obtained from U. Heberlein (UCSF). $Rdl^{RNAi4\text{-}5SE}$ and $Rdl^{RNAi8\text{-}10}$*/TM6B* were obtained from R. Davis (Scripps Research Institute, Florida). rut^{2080}, rut^{1}, dnc^{1}, ry^{506}, *Berlin,* $rut^{RNAiJF02361}$, $dnc^{RNAiHMC03573}$, *UAS-TrpA1,* $Lcch3^{RNAiJF02159}$*/TM3*, $Grd^{RNAiJF03268}$, $GABA\text{-}BR1^{RNAiJF02989}$*/TM3*, $GABA\text{-}BR3^{RNAiJF02271}$ lines were obtained from the Bloomington Stock Center (Bloomington, Indiana). $Lcch3^{RNAi\text{-}37409}$, $Grd^{RNAi\text{-}5329}$, $GABA\text{-}BR1^{RNAi\text{-}101440}$, $GABA\text{-}BR2^{RNAi\text{-}1784}$, $GABA\text{-}BR2^{RNAi\text{-}1785}$ and $GABA\text{-}BR3^{RNAi\text{-}108036}$ were obtained from the Vienna *Drosophila* RNAi Center (Vienna, Austria). *DaGsw-GAL4* was obtained from Marc Tatar (Brown University). *PsnB3/TM6C* and *PsnC4/TM6C* mutants were obtained from T. Jongens (University of Pennsylvania).

Sleep

Sleep was assessed as previously described [S1, S2]. Briefly, flies were placed into individual 65 mm tubes and all activity was continuously measured through the Trikinetics Drosophila Activity Monitoring System (www.Trikinetics.com, Waltham, Ma). Locomotor activity was measured in 1-minute bins and sleep was defined as periods of quiescence lasting at least 5 minutes.

Sleep Deprivation

Sleep deprivation was performed as previously described [S2]. Briefly, flies were placed into individual 65 mm tubes and the sleep-nullifying apparatus (SNAP) was used to sleep deprive these flies for 12 hours during the

dark phase (lights out to lights on). Sleep homeostasis was calculated for each individual as a ratio of the minutes of sleep gained above baseline during the 48 h of recovery divided by the total min of sleep lost during 12 h of sleep deprivation.

Arousal Thresholds

Arousal thresholds were calculated as done previously [S3]. Flies housed individually in glass tubes were probed hourly across 24h, with a succession of vibrational stimuli of increasing strength, from 0 to 1.2g. Each stimulus consisted of 5 pulses of 200ms, and was delivered in 0.24g increments 15 seconds apart. The arousal threshold for each individual fly was calculated by assigning the weakest vibration intensity (g) required to elicit a response (walking at least half the length of the glass tube) in quiescent flies that had not shown activity in at least the preceding minute. The median value and distribution of arousal thresholds (g value) was then calculated for each strain. Video tracking methods were used to track fly movement [S3].

Short-term memory

Short-term memory (STM) was assessed by Aversive Phototaxic Suppression (APS) as previously described [S1, S2]. The experimenters were blinded to condition. In the APS, flies are individually placed in a T-maze and allowed to choose between a lighted and darkened chamber over 16 trials. Flies that do not display phototaxis during the first block of 4 trials are excluded from further analysis [S1, S4]. During 16 trials, flies learn to avoid the lighted chamber that is paired with an aversive stimulus (quinine/ humidity). The performance index is calculated as the percentage of times the fly chooses the dark vial during the last 4 trials of the 16 trial test. In the absence of quinine, where no learning is possible, it is common to observe flies choosing the dark vial once during the last 4 trials in Block 4 [S1]. In contrast, flies never choose the dark vial 2 or more times during block 4 in the absence of quinine[S1]. Thus, STM is defined as two or more photonegative choices in Block 4. For STM experiments following a 12 h sleep deprivation, the deprivation continued until evaluation in the APS. All flies were tested in the morning. Power analysis using G*Power calculates a Cohen's d of 1.8 and indicates that

eight flies/group are needed to obtain statistical differences [S1]. To systematically evaluate the effects of sleep on subsequent performance we obtained memory mutants, as identified by aversive olfactory conditioning, that fit into the classes described by [S5]. Note that since the APS and olfactory conditioning are very different assays, and since different training protocols within olfactory conditioning produce different phenotypes [S6], the phenotype observed in the APS for a given mutant allele may not phenocopy the exact results originally reported using a single specific training protocol for olfactory conditioning.

Photosensitivity

Photosensitivity was evaluated as previously described[S1]. Briefly, flies were put in the T-maze over 10 trials in the absence of filter paper. The lightened and darkened chambers appeared equally on both the left and right. The photosensitivity index (PI) is the average of the scores obtained for 5-6 flies ± s.e.m..

Quinine sensitivity

Quinine sensitivity index (QSI) was evaluated as previously described[S1, S2]. Briefly, flies were individually placed at the bottom of a 14 cm transparent cylindrical tube which was uniformly lighted and maintained horizontal after the introduction of the animal. Each half of the apparatus contained separate pieces of filter paper which could be wetted with quinine or kept dry. The QSI was determined by calculating the time in seconds that the fly spent on the dry side of the tube when the other side had been wetted with quinine, during a 5 min period.

Courtship Conditioning

Training for 4–8 day old males was based on previously described methods[S7]. The males were exposed to pheromonally-feminized *Tai2* males in either a training protocol consisting of three one-hour training sessions, each separated by one hour, or a single training protocol consisting of one three-hour training session. Long-term memory was tested forty-eight hours after the beginning of training, when trained and naive males were

exposed to *Tai2* males for a 10-minute testing period (n=16-30 flies/condition). The Courtship Index (CI) is defined as the percent of time that each subject fly spends in courtship behavior during the 10-minute testing period. The CIs were subjected to an arcsine square root transformation to approximate normal distribution as described in [S8]. Data are presented as a Performance Index (PI), where $PI=(CI_{average\text{-}naive}-CI_{Trained})/CI_{average\text{-}naive}$ X100); PIs were evaluated using the Kruskal-Wallis test. The experimenters were blinded to condition. For the single training experiments using THIP-induced sleep, naïve and trained males were fed 0.1 mg/ml THIP (0.1T) or SKF97541 (40µM) for 4 h after training and were returned to vehicle afterwards. Vehicle-fed flies were maintained on vehicle throughout the experiment. For the 3-training session experiments with THIP-induced sleep, naïve males were fed 0.1T for 48 h prior to training. THIP-fed flies were removed from THIP 1 h prior to and during training (half of the 0.1T-fed flies were trained) and then returned to 0.1T for 24 h post-training. Vehicle-fed flies were maintained on vehicle throughout the protocol.

QPCR

QPCR were performed as previously described [S2, S7]. Briefly, total RNA was isolated from ~20 fly heads with Trizol (Invitrogen, Carlsbad, CA) and DNAse I digested. cDNA synthesis was performed in triplicate using Superscript III (Invitrogen, Carlsbad, CA), according to manufacturer protocol. In order to evaluate the efficiency of each reverse transcription, equal amounts of cDNA were used as a starting material to amplify RP49 as previously described. cDNA from comparable reverse transcription reactions were pooled and used as a starting material to run three QPCR replicates. Expression values for RP49 were used to normalize results between groups.

Western blot

Sixteen fly brains per group were dissected and homogenized in 15µl cell lysis buffer (10 mM Tris, pH 8.5/8 M urea/4% CHAPS /5 mM magnesium acetate) with 1x protease inhibitor cocktail (Roche). Lysates were normalized for proteins (Bradford protein assay-Biorad laboratories) and 1µg of protein was mixed with

sample buffer (4% SDS, 20% glycerol, 10% 2-mercaptoethanol, 0.004% bromophenol blue and 0.125 M Tris HCl, pH approx. 6.8 - Sigma Aldrich, St Louis, MO) to a total of 12µl. The samples were then heated to 100° Celsius for 5 minutes and then centrifuged at max speed for 3 minutes and loaded on a gradient gel (4-15% TGX (Biorad)). Gel was run at 80v for 1 hour and 100v until the samples run off the gel and then transferred to PVDF membrane at 4° Celsius at 100v for 1.5 hours. Blot was probed 1:4000, mouse anti-DLG (4F3, Developmental Studies Hybridoma Bank,University of Iowa), mouse anti-TUBULIN antibody (E7-βTUBULIN) – (Developmental Studies Hybridoma Bank,University of Iowa) 1:1000 followed by anti-rabbit secondary 1:1000 and anti-mouse secondary 1:1000 (Sigma Aldrich) respectively. Blot was visualized using ECL HRP substrate (Thermoscientific) and a Biorad chemiluminescence detector and quantified using ImageJ software (NIH). After background correction optical densities were calculated and normalized (by dividing with the within-lane tubulin signal used as loading control). The protein/tubulin ratio of the treated samples was compared to the control lane in the same gel to measure relative increase. Statistical analysis was done using Student's t-test for two-group comparisons.

Electrophysiology

Brain recordings were performed as described previously[S9]. Briefly, local field potentials (LFPs) were sampled at 300 Hz as a voltage differential from two glass electrodes inserted into the fly brain, one in each hemisphere. Flies were filmed, and sleep was identified after 5 min immobility as described previously[S9]. Fourier analyses of LFPs were performed using MATLAB software (MathWorks) to calculate power across all frequencies 1-100Hz. LFP data for awake or sleeping flies following THIP feeding were normalized to earlier control LFP activity in the same flies after they were fed food without THIP. Food ingestion (with or without THIP) was observed for each fly used in the dataset.

Lifespan

Lifespan was evaluated as previously described[S10]. Briefly, 3-day old flies were placed into vials n=10/vial with food or 0.1mg/mL of THIP and monitored until all flies were dead. Vials were changed every 4 days.

Pharmacology

THIP was administered at dosages of 0.025 mg/mL, 0.05 mg/mL, 0.1 mg/mL and 0.5mg/mL in standard fly food. Flies were maintained on the drug for the durations described in the text during which time sleep was monitored. Flies were removed from THIP one hour prior to being tested for short-term memory and one hour prior to being trained for courtship conditioning. Ethanol (10%), SKF97541 (40µM), reserpine (20µM) and 1,4-butanediol (2%) were dissolved in standard fly food.

Statistics

All comparison were done using a Student's T-test or, if appropriate, ANOVA and subsequent planned comparisons using modified Bonferroni test unless otherwise stated. Note that a significant omnibus-F is not a requirement for conducting planned comparisons [S11]. All statistically different groups are defined as *P < 0.05.

AUTHOR CONTRIBUTIONS

SD, YS, VA, JD, MK, LK, DE, ZK, RWS, BVS and PJS performed experiments. SD, BVS, RWS and PJS oversaw experiments, analysis, and project direction. SD, BVS, and PJS planned and designed experiments and wrote the paper.

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