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Supplemental Information

Identification of a Neural Circuit that Underlies the Effects of Octopamine on Sleep:Wake Behavior

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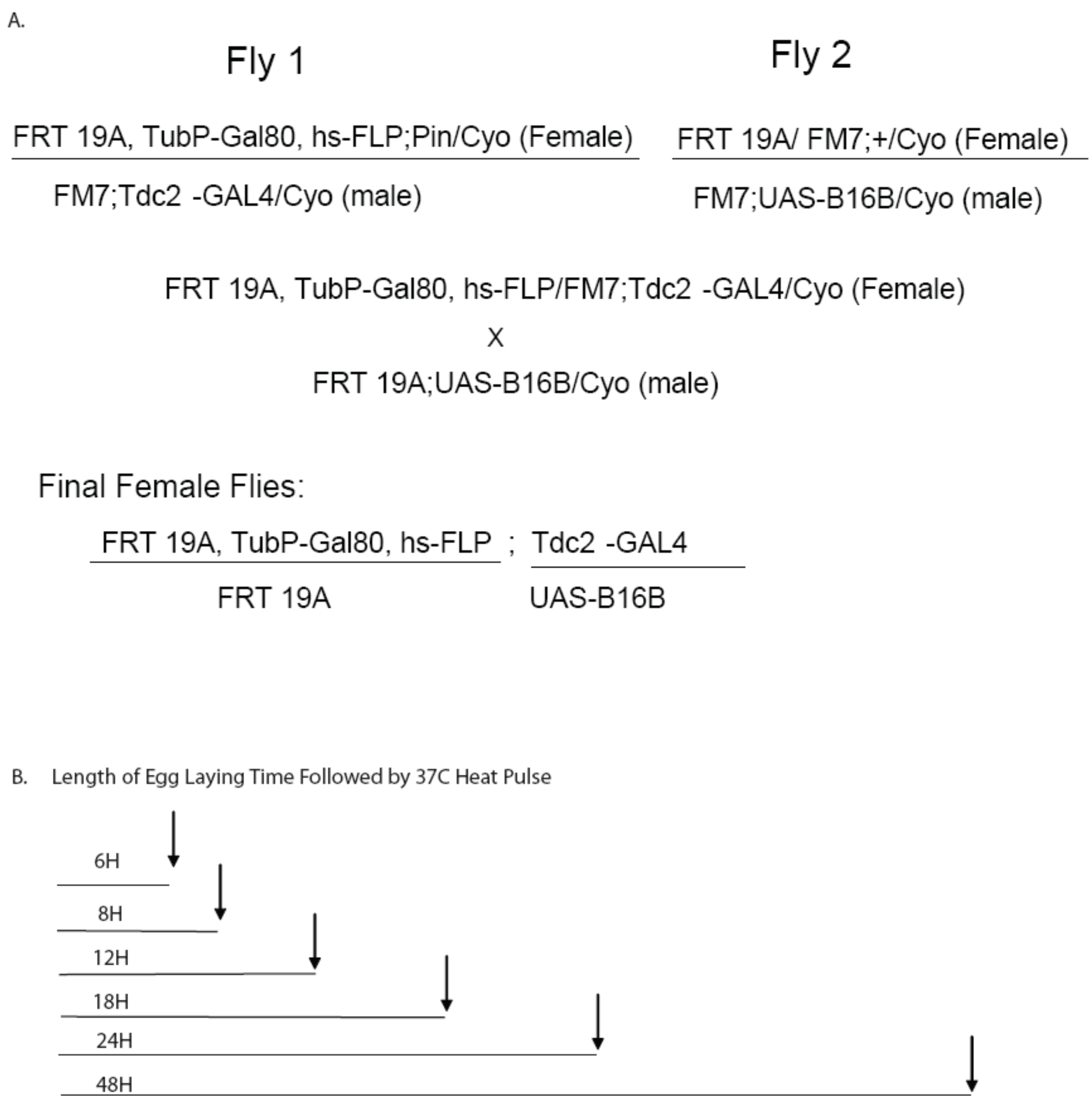
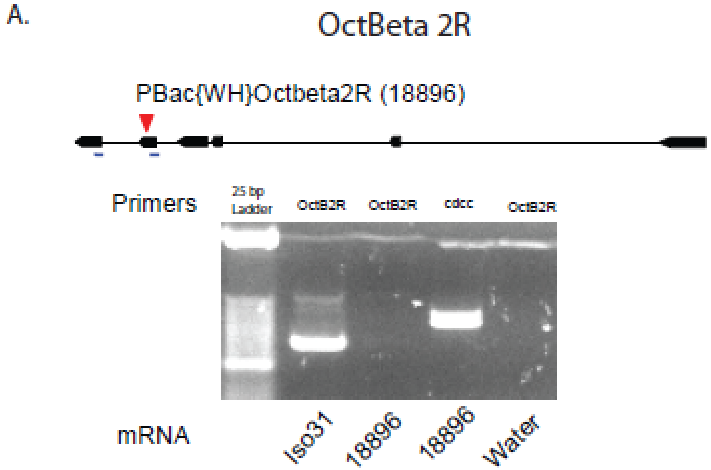
Supplemental Figure 1

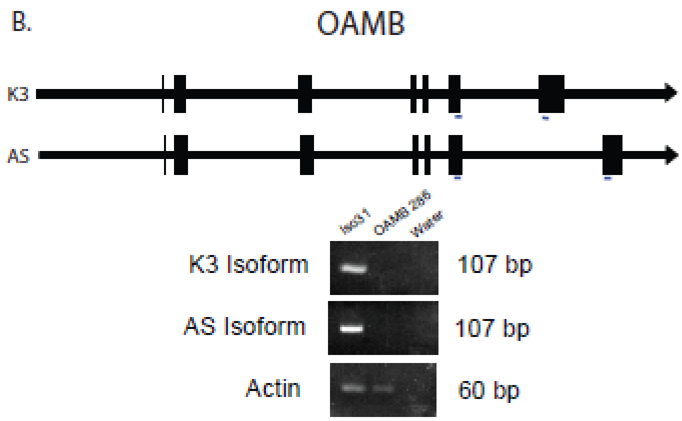


Supplemental Figure 1 : MARCM Methods (related to Figure 1).

A. Outline of the crosses performed to generate the female flies for MARCM analysis. We used flies with the FRT (Flp recombination target) site as well as the FLP recombinase and the Gal80 driven by a tubulin promoter on the 1st chromosome,. The second chromosome carried the *Tdc2*-GAL4 and UAS-*NaChBac* transgenes. B. Outline of the heat shock regime to induce expression of FLP recombinase. Parents of the final offspring were allowed to lay eggs for the times indicated before being removed from the vial; eggs/larvae were then heat pulsed at 37 degrees. Note that each population was only subjected to one treatment; induction of the recombinase at different stages in different populations resulted in a wide range of "mosaic" progeny, from those that expressed the sodium channel in many cells to those that showed expression in only one cell.

Supplemental Figure 2

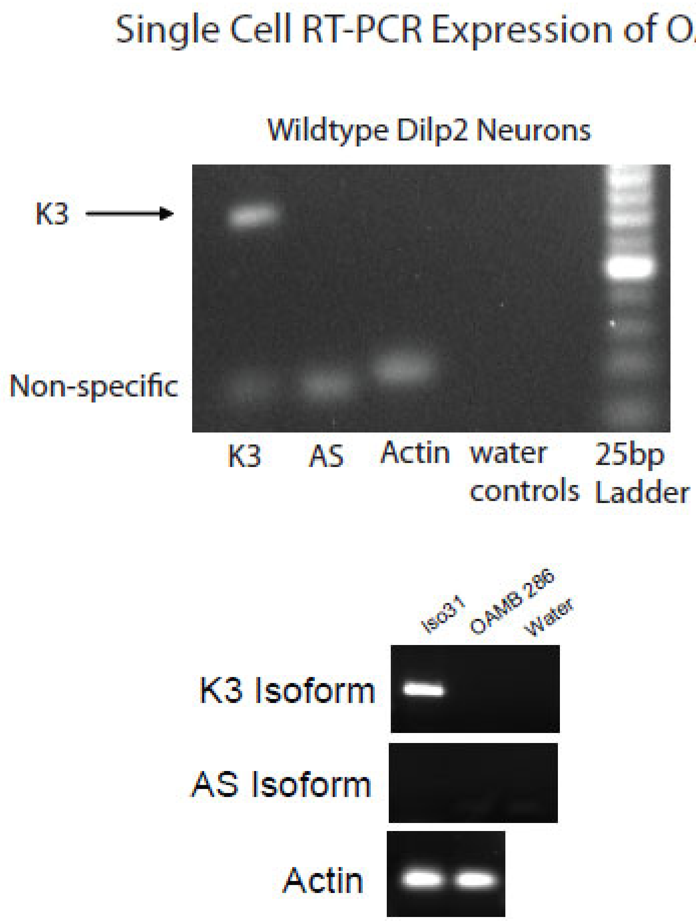




Supplemental Figure 2 (related to Figure 6). Molecular analysis of the OAMB and OctB2 receptors

A. Schematic of the OctB2R gene showing the P-element insertion (red arrow). Primers were designed to flank this insert and to span an exon (blue lines under exon) and then RT-PCR was conducted to assay expression of OctB2R. Representative gel showing lack of a product in OctB2R flies as compared to the control lane, implying that these flies are mutant for the OctB2R gene. B. Verification of the OAMB 286 mutant. RT-PCR was run on control flies and OAMB 286 mutants using the new K3 and AS primers to both verify primer specificity and to verify that OAMB 286 is a true null.

Supplemental Figure 3

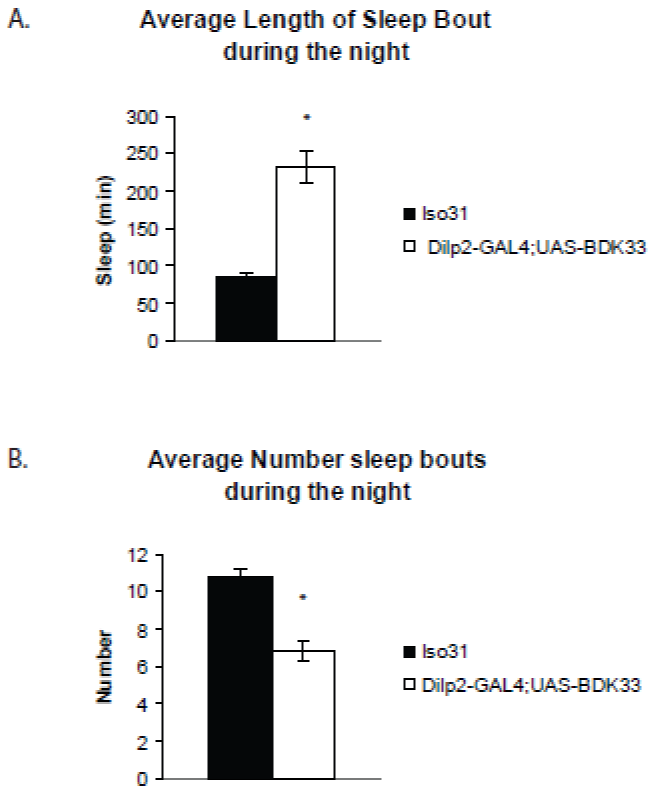


Supplemental Figure 3 (related to Figure 6). The OAMB receptor is expressed in *Dilp2* neurons

Single cell RT-PCR of control flies showing that only the K3 isoform is present in *Dilp2* producing neurons. B. Representative gel for single cell RT-PCR of control

Dilp2 neurons and OAMB 286 mutant *Dilp2* neurons. In the OAMB 286 mutant the K3 isoform is no longer present.

Supplemental Figure 4.



Supplemental Figure 4 (related to Figure 2). Expressing PkaR in *Dilp2* neurons consolidates sleep.

A. The average length of sleep bouts is significantly longer in flies expressing UAS-BDK33 (PkaR) in *Dilp2* neurons using the *Dilp2*-GAL4 driver. Control flies (Iso31) have an average sleep bout length at night of 83.86 ± 7.27 minutes while the Dilp2-GAL4;UAS-BDK33 flies have an average night-time bout length of

233.1 ± 21.21 minutes. Sleep bout length is plotted as mean ± s.e.m. Asterisk= $P < 0.01$ by 2-way Anova.

B. The average number of sleep bouts is significantly less in flies expressing UAS-BDK33 (PkaR) in *Dilp2* neurons using the *Dilp2*-GAL4 driver. Control flies (Iso31) have on average 10.6 ± 0.47 sleep bouts at night while the *Dilp2*-GAL4;UAS-BDK33 flies have 6.85 ± 0.56 sleep bouts. Sleep bout number is plotted as mean ± s.e.m. Asterisk= $P < 0.01$ by 2-way Anova.

Supplemental Table 1 (related to Figure 1): Sleep data for flies generated through MARCM

	Total Sleep	Daytime Sleep	Nighttime Sleep	N
ASM	663.25±36.5*	155.49±25.22*	476.13±39.77*	12
ASM single cell	870.45±25.59*	279.18±33.39*	598.96±17.3	11
VL	1007.18±28.29	372.49±27.97	587.68±21.56	21
PB1 and PB2	1030.21±24.47	393.72±20.47	625.80±11.15	23
AL	1021.13±20.3	381.22±19.66	607.44±20.31	28
VUM 1,2	1016.68±19.91	379.17±16.57	612.61±12.04	47
Thorax	1045.76±23.57	408.24±20.66	606.75±18.74	33
No cells	1025.86±13.45	391.45±11.50	616.27±8.23	84
PSM	1109.92±15.64	458.75±15.76	664.87±15.08	12

Supplemental Table 1. Sleep data for flies generated through MARCM Table depicts total sleep, daytime sleep and nighttime sleep for each group of TDC2 positive cells.

Supplemental Table 2 (related to Figures 2 and 7): Effects of octopamine on sleep in different fly lines

GAL4 Line	Nighttime Sleep	Sleep following 10mg/ml Octopamine		N
Control (Iso31)	623.09 ± 11.27	489.5 ± 22.21	*	64
MBGS	661.21 ± 14.2	483.86 ± 21.45	*	28
H24	629.42 ± 19.64	503.17 ± 24.77	*	24
17d	597.92 ± 11.7	493.71 ± 21.46	*	48
c747	675.35 ± 8.79	559.35 ± 32.51	*	40
c309	699.75 ± 3.46	619.14 ± 15.09	*	56
1366	646.70 ± 21.33	395.00 ± 40.25	*	20
MJ63	648.64 ± 15.51	500.71 ± 19.89	*	32
c507	628.91 ± 11.46	474.91 ± 23.43	*	44
104y	572.96 ± 14.69	459.35 ± 31.38	*	46
D42	717.25 ± 2.75	636.25 ± 24.18	*	30
mai301	650.20 ± 12.63	433.73 ± 32.11	*	30
50Y	624.33 ± 8.75	519.8 ± 33	*	32
30y	663.00 ± 28.00	627.00 ± 8.00		16
Sep-54	705.38 ± 7.78	683.5 ± 11.54		32
201y	629.16 ± 13.97	637.26 ± 11.69		62
Dilp2	642.57 ± 9.18	645.85 ± 12.4		32
elavGS	665.41 ± 7.00	666.77 ± 11.09		44
Kurs58	609.86 ± 9.07	613.96 ± 18.14		32

	Nighttime Sleep	Nighttime sleep following 10mg/ml octopamine		N
w1118	598.5 ± 9.72	490.57 ± 30.35	*	32
OAMB 286	671.96 ± 7.09	635.46 ± 11.85		30
OAMB 286 Rescue	544.43 ± 27.0	387.0 ± 41	*	32

Supplemental Table 2 (related to Figures 2 and 7): Effects of octopamine on sleep in different fly lines The first part of the table shows effects of octopamine on sleep in flies expressing PkaR under the control of different GAL4 drivers. The bottom part shows effects on octopamine receptor mutants.

Supplemental Table 3 (related to Figures 3 and 6): Sleep in octopamine receptor mutants and in flies with altered excitability of *Dilp2* neurons

	Total Sleep	Activity per waking minute	Daytime Sleep	Nighttime Sleep	sleep bout length day	sleep bout length night	sleep bout number day	sleep bout number night
w1118;+;+ F	956.0 ± 14.27	1.85 ± 0.12	342.9 ± 9.22	613.60 ± 6.78	49.41 ± 3.82	141.86 ± 13.53	8.96 ± 0.75	5.6 ± 0.38
w1118; +; + M	977.77 ± 14.62	2.53 ± 0.15	400.44 ± 8.54	577.33 ± 8.35	66.08 ± 8.36	99.51 ± 7.76	8.17 ± 0.50	7.33 ± 0.51
w1118;Dilp2-GAL4; + F	966.19 ± 19.08	1.05 ± 0.03*	349.68 ± 13.90	616.50 ± 8.39	21.16 ± 1.88*	126.51 ± 13.61*	21.03 ± 0.82*	9.24 ± 0.55*
w1118;Dilp2-GAL4; + M	933.34 ± 17.13*	1.74 ± 0.05*	386.43 ± 14.06	546.91 ± 6.81*	48.27 ± 5.78*	87.34 ± 8.22	11.40 ± 0.54*	9.70 ± 0.76*
w1118;; NaChBac F	884.62 ± 29.28*	1.29 ± 0.07*	343.75 ± 30.65	540.88 ± 18.49*	33.61 ± 2.65*	52.87 ± 5.83*	12.02 ± 0.89*	14.41 ± 0.89*
w1118;; NaChBac M	1005.21 ± 9.81	2.8 ± 0.05*	440.80 ± 5.35*	564.41 ± 8.76	169.63 ± 17.06*	121.34 ± 7.36*	3.98 ± 0.35*	5.62 ± 0.38
w1118;Dilp2; NaChBac F	784.33 ± 21.92*†	1.33 ± 0.08*†	273.83 ± 18.32*†	510.50 ± 9.67*†	24.21 ± 2.02*	39.13 ± 2.48*†	12.44 ± 1.02*†	15 ± 0.75*†
w1118;Dilp2; NaChBac M	779.45 ± 22.30*†	2.41 ± 0.2	373.64 ± 7.67	405.81 ± 18.77*†	61.31 ± 4.48	54.83 ± 4.34*†	7.17 ± 0.45	8.5 ± 0.48
w1118; 3xEKOII F	1000.86 ± 12.90*	1.33 ± 0.05*	409.04 ± 12.83*	591.82 ± 7.98	31.49 ± 2.51*	151.14 ± 23.52	15.79 ± 0.98*	7.82 ± 0.89*
w1118; 3xEKOII M	1059.03 ± 10.69*	1.63 ± 0.04*	428.10 ± 8.54	630.92 ± 6.24*	69.17 ± 5.39	262.18 ± 22.83*	8.44 ± 0.59	3.88 ± 0.36*
w1118; Dilp2/3xEKOII F	1135.19 ± 7.99*†	1.04 ± 0.09*	560.73 ± 5.66*†	574.46 ± 5.53*†	71.34 ± 4.62*†	135.39 ± 15.15	9.24 ± 0.59	5.95 ± 0.45
w1118; Dilp2/3xEKOII M	1210.88 ± 8.36*†	1.54 ± 0.07*†	560.31 ± 6.96*†	650.56 ± 3.30*†	115.59 ± 8.44*†	224.88 ± 17.82*†	5.81 ± 0.35*†	3.8 ± 0.29*†
w1118 F	848.31 ± 40.41	1.87 ± 0.04	253.29 ± 30.34	595.02 ± 13.46	30.86 ± 4.36	72.63 ± 11.30	10.4 ± 0.78	11.68 ±1.13
w1118 M	970.68 ± 9.48	2.35 ± 0.05	446.54 ± 6.64	524.13 ± 6.66	55.38 ± 3.25	94.58 ± 6.91	12.53 ± 0.68	9.99 ± 0.63
OAMB 286 F	1169.52 ± 36.20*	2.60 ± 0.27*	490.37 ± 35.34*	679.15 ± 6.59*	24.20 ± 2.45	142.48 ± 27.21*	21.76 ± 1.53*	7.39 ±1.11*
OAMB 286 M	1215.29 ± 20.63*	2.15 ± 0.18	551.38 ± 14.46*	663.81 ± 7.37*	43.25 ± 4.94*	133.39 ± 17.71*	15.61 ± 1.51*	7.54 ± 0.95
OAMB 584 F	1165.13 ± 26.42*	2.37 ± 0.17*	478.33 ± 22.89*	686.79 ± 5.48*	22.74 ± 1.78	126.56 ± 25.16*	22.50 ± 1.16*	9.20 ± 1.21
OAMB 584 M	1068.38 ± 29.40	2.72 ± 0.12	454.62 ± 21.50	613.85 ± 12.48	38.60 ± 4.54*	72.04 ± 12.74	14.41 ± 1.19*	12.10 ± 1.06*
UAS-K3;;OAMB286/OAMB286 F	1088.18 ± 24.45	2.0 ± 0.16	411.23 ± 23.83	676.14 ± 5.12	23.62 ± 1.93	242.07 ± 37.79	19.96 ± 1.38	5.89 ± 0.89
UAS-K3;;OAMB286/OAMB286 M	1103.90 ± 7.05	1.43 ± 0.04	476.89 ± 6.05	627.01 ± 4.99	45.05 ± 3.50	228.47 ± 23.33	13.22 ± 0.60	4.97 ± 0.41
w1118;Dilp2-GAL4;OAMB 286/OAMB286 F	1127.719 ± 25.58	2.10 ± 0.09	479.72 ± 23.65	648.0 ± 56.9	39.01 ± 4.7	174.0 ± 25.0	13.75 ± 1.04	6.11 ± 1.1
w1118;Dilp2-GAL4;OAMB 286/OAMB286 M	1047.62 ± 19.95	2.38 ± 0.21	455.26 ± 13.18	592.35 ± 11.60	25.76 ± 1.69	80.94 ± 9.37	19.74 ± 0.88	10.52 ± 0.93

UAS-K3;Dilp2-GAL4;OAMB286/OAMB286 F	945.0 ± 16.73**	1.5 ± 0.05**	330.13 ± 13.44**	614.53 ± 8.78**	37.14 ± 5.68*	167.02 ± 20.84*	18.37 ± 0.77*	10.63 ± 0.95**
UAS-K3;Dilp2-GAL4;OAMB286/OAMB286 M	953.98 ± 20.54**	2.03 ± 0.08**	426.32 ± 18.06*	527.66 ± 11.41**	49.63 ± 5.69*	142.42 ± 19.99**	11.5 ± 0.68*	5.86 ± 0.44**
Iso31 F	881.17 ± 28.45	1.46 ± 0.03	311.73 ± 21.57	569.43 ± 10.95	31.49 ± 1.70	104.35 ± 7.44	12.8 ± 0.43	8.94 ± 0.40
Iso31 M	952.59 ± 17.60	2.03 ± 0.06	388.72 ± 12.20	563.87 ± 9.03	63.90 ± 5.45	119.89 ± 10.48	8.21± 0.54	6.58 ± 0.48
OctB2R (18896) F	765.58 ± 25.60*	1.35 ± 0.03*	210.91 ± 20.02*	554.67 ± 12.08	22.58 ± 1.61*	68.58 ± 5.59*	12.18 ± 0.52	10.79 ± 0.44*
OctB2R (18896) M	970.84 ± 20.89	2.11± 0.07	435.55 ± 12.36*	535.29 ± 12.17	90.02 ± 4.69*	93.54 ± 7.39	5.96 ± 0.28*	7.52 ± 0.54

Supplemental Table 3 (related to Figures 3 and 6): Sleep in octopamine receptor mutants and in flies with altered excitability of *Dilp2* neurons

In the tables above Dilp2 refers to *Dilp2*-GAL4, NaChBac to UAS-*NaChBac* and 3xEko to UAS-*EkoII*;UAS-*EkoI*. The w1118 background for OAMB 286 and OAMB 584 is different from the isogenic w1118 strain, termed Iso31, we used in other experiments. All tables depict mean ± SEM *= p<.01 relative to wildtype control line (w1118 or Iso31, as the case may be). All "†" for w1118;NaChBac, w1118;3xEkoII, w1118;Dilp2/NaChBac and w1118;Dilp2/3xEkoII are relative to each respective GAL4 and UAS control. The "**" for rescue experiments indicate the rescue is significantly different from each control- wild type, mutant and mutant with either UAS or GAL4 alone. Dilp2/NaChBac females are significantly different from all controls for total sleep, daytime sleep, nighttime sleep, sleep bout length night and sleep bout number night. Dilp2/NaChBac males are significantly different from all controls for total sleep, daytime sleep, nighttime sleep, and sleep bout length night. Dilp2/3xEkoII females are significantly different from all controls for total sleep, daytime sleep, and daytime sleep bout length. Dilp2/3xEkoII males are significantly different from all controls for total sleep, daytime sleep, nighttime sleep, daytime sleep bout length, nighttime sleep bout length, daytime sleep bout number and nighttime sleep bout number.