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A genetic screen to assess Dopamine Receptor (DopR1) dependent sleep regulation in *Drosophila*.

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Abstract

Sleep is an essential behavioral state of rest that is regulated by homeostatic drives to ensure a balance of sleep and activity, as well as independent arousal mechanisms in the central brain. Dopamine has been identified as a critical regulator of both sleep behavior and arousal. Here we present results of a genetic screen that selectively restored the Dopamine Receptor (*DopR/DopR1/dumb*) to specific neuroanatomical regions of the adult *Drosophila* brain to assess requirements for DopR in sleep behavior. We have identified subsets of the mushroom body that utilize DopR in daytime sleep regulation. These data are supported by multiple examples of spatially restricted genetic rescue data in discrete circuits of the mushroom body, as well as immunohistochemistry that corroborates the localization of DopR protein within mushroom body circuits. Independent loss of function data using an inducible RNAi construct in the same specific circuits also supports a requirement for DopR in daytime sleep. Additional circuit activation of discrete DopR$^+$ mushroom body neurons also suggests roles for these subpopulations in sleep behavior. These conclusions support a new separable function for DopR in daytime sleep regulation within the mushroom body. This

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daytime regulation is independent of the known role of DopR in nighttime sleep, which is regulated within the Fan Shaped Body. This study provides new neuroanatomical loci for exploration of dopaminergic sleep functions in *Drosophila*, and expands our understanding of sleep regulation during the day versus night.

Introduction

Identifying the cellular and molecular mechanisms that control arousal and sleep is an important pursuit for understanding rest homeostasis (Shaw *et al.*, 2000), as well as facilitating a deeper understanding of sleep disorders in humans (Donelson & Sanyal, 2015). In *Drosophila melanogaster*, sleep has been characterized as consolidated periods of rest marked by a decreased responsivity to arousing stimuli and represented as a homeostatic drive that requires fulfillment of rest for optimal performance in cognitive and innate tasks (Dissel *et al.*, 2015). Sleep in *Drosophila* can be divided into multiple behavioral dimensions for investigation of potentially separable aspects of sleep behavior, such as onset of sleep, duration, number of sleep bouts, and average duration of individual sleep bouts. Furthermore, both genetic and environmental factors, such as temperature and light, can differentially affect parameters of *Drosophila* sleep in the day versus night period (Ishimoto *et al.*, 2012, Parisky *et al.*, 2016).

Dopamine has been identified as a key regulator of sleep in *Drosophila* (Andretic & Hirsh, 2000, Kume *et al.*, 2005, Lebestky *et al.*, 2009, Sitaraman *et al.*, 2015b), however the cellular loci of presynaptic and post-synaptic control are

complex. Previously, a requirement for the Type I Dopamine Receptor, *DopR/DopR1/dumb*, was localized to the dorsal fan-shaped body, as well as an absence of DopR function in the Mushroom Body with regards to observed sleep behavior (Ueno *et al.*, 2012). This contrasts with a previously known requirement for neural activity in the mushroom body in sleep behavior (Joiner *et al.*, 2006, Pitman *et al.*, 2006), however it could be consistent with independent control of sleep behavior in the mushroom body that is not subject to direct dopaminergic regulation. Alternatively, multiple groups have recently ascribed micro-circuitry, or discrete, functional subsets of neurons within the brain, as having opposing roles or separable functions within a larger structure, and this may also explain differences in broad versus specific manipulations within a given neuroanatomical structure or class of neurons (Seidner *et al.*, 2015, Sitaraman *et al.*, 2015a).

Given the known regions of high DopR expression in discrete brain structures such as the central complex and mushroom body (Kim *et al.*, 2003, Kong *et al.*, 2010, Lebestky *et al.*, 2009), and the potential for less conspicuous but functionally relevant DopR in other brain regions, we sought to utilize the dominant, haploinsufficient sleep phenotype of the UAS piggyBac insertional mutation, $DopR^{f02676}$/+ heterozygous animals as a sensitized screening background for identifying new neural circuits that use DopR in regulating sleep behavior (Figure 1). This genotype has proven to be a useful tool in characterizing Dopamine signaling and the circuit-based requirements for DopR in multiple *Drosophila* behaviors (Kim *et al.*, 2007, Kong *et al.*, 2010, Lebestky *et al.*, 2009). Previous data suggests a clear excess of sleep for both $DopR^{f02676}$/+ and $DopR^{f02676}$/ $DopR^{f02676}$

mutant animals (Lebstky et al. 2009). In the following study, we utilized many neuronal GAL4 lines as well as lines from the Flylight collection of Gal4 lines (Jenett *et al.*, 2012) to restore DopR in discrete circuits of the brain and CNS and assess changes to sleep behaviors. The highly anatomically characterized Flylight Collection allows for improved specificity for individual Gal4 lines to allow for deeper investigation of DopR requirements in sleep behavior. Here we report the screening procedures and results for identifying new neural circuits that require *DopR* for normal daytime sleep behavior. Furthermore, this day regulation is shown to be separable from the existing known requirement for *DopR* in nighttime sleep behavior.

Materials and Methods

Genetic Screen: F1 Crosses for behavioral analysis

The dominant hypomorphic mutant allele *DopR*^{f02676} contains a *piggy-Bac* element insertion with a UAS sequence in the first intron (Exelixis Collection at Harvard medical School). This allele displays haplo-insufficiency for certain behaviors, as we and others observe a loss of mRNA (50-60%) and reductions in protein expression (observed via immunohistochemistry) in the adult brain (Lebestky et al. 2009, Kong et al. 2010). When crossed to Gal4 stocks, the resultant genotype (*Gal4/+;DopR*^{f02676}*/+)* will produce a truncated, but functional version of the protein only where the Gal4 is expressed (Lebestky et al. 2009, Kong et al. 2010, and Kim et al. 2007). It is currently unknown whether the *DopR*^{f02676} allele, alone in the absence of Gal4, also produces alternatively spliced or dominant negative forms

of the protein. The *DopR*^{f02676} allele was backcrossed into the *Canton S* (*CS*) wild-type background for six generations (as described in Lebestky et al. 2009). Gal4 lines were acquired from the Kaiser collection (Armstrong *et al.*, 1998) and the Janelia Farm Flylight collection at Bloomington Drosophila Stock Center (BDSC; Bloomington, IN). Expression data including high resolution z-stack projections and movies for all Flylight collection Gal4 lines are made available to the entire *Drosophila* community by HHMI Janelia Farm for review at the following URL: [http://flweb.janelia.org/cgi-bin/flew.cgi]. All lines used in our study can be observed by selecting "Line or associated gene (adult)" in the "Search Lines" function and inserting the genotype into the search window (R59H05 for example). Fly stocks were maintained at 18°C, and crosses were grown at 25°C. All flies were kept in 12:12 light:dark cycle conditions. Flies were reared on Bloomington recipe fly food. Fly strains generated in our lab are available for distribution and data sets for sleep parameters are available upon request.

For F1 screening, *DopR*^{f02676} homozygous virgins or CS virgin females were crossed with homozygous *Gal4* males, and F1 males were selected. Genetic controls for *Gal4* flies acquired from Janelia Farm collection consists of recombination of control fly line that is "empty" at the recombination attp2 site at 68A4 on 3L (Jenett *et al.*, 2012, Pfeiffer *et al.*, 2008). Controls for Gal4 lines of the Kaiser collection (Armstrong *et al.*, 1998) were performed by crossing CS virgins flies to males with the Gal4 enhancer trap c561, which has a P-element insert on the X chromosome and is therefore not transmitted to F1 hybrid males within the given crossing

scheme, but autosomes represented in the Kaiser collection are present in the F1 hybrid.

Determination of whether a given Gal4-rescue genotype is regarded as a "fail" versus "rescue" in Tables 1 and 2 is based on the statistical comparison for total sleep and sleep duration between the Gal4/+ and DopR/Gal4 genotypes (See statistics below). If these are not significantly different from each other and they are significantly different from DopR/+, the potential rescue condition is validated. If the rescue condition is not significantly different from the DopR/+ and is significantly different from the Gal4/+ genotype, the rescue genotype is regarded as a "fail." For rare cases where Gal4/+ is not significantly different from DopR/+ for sleep parameters, indicating confounds due to insertional variation or genetic background, the line is also regarded as a "fail."

Sleep Behavior

To assess sleep and activity of individual flies, the Drosophila Activity Monitoring System (DAMS; Trikinetics, Waltham, MA) was used. For each F1 progeny genotype, approximately 40 3-5 day old males were collected in batches of 10 animals and stored overnight at 25°C and maintained on the same 12:12 Light/Dark cycle as for the genetic cross and stocks. The next day, 32 individual males were transferred via aspiration into DAMS monitor tubes that contained standard food. Tubes were then loaded into a DAMS monitor, 32 tubes of each genotype per monitor. Monitors were kept in DigiTherm® CircKineticsTM incubators (Tritech Research) at 25°C with a 12:12 LD cycle. Raw activity data was

processed with the DAMFileScan program (Trikinetics) to sort the data into one and 30 minute bins. The output files were analyzed using Sleep and Circadian Analysis Matlab Program (SCAMP) developed in the Griffith Lab (Waltham, MA).

TrpA1 Neuronal Activation Experiments

Individual Gal4 stocks were crossed to UAS-TrpA1 and reared at 23 degrees. Transheterozygous F1 progeny (Gal4 / UAS-TrpA1) were collected and reared in a similar manner as described above. 32 individual animals of the three genotypes: Gal4/+ , UAS-TrpA1/+, and Gal4/UAS-TrpA1 were places in DAMS monitors at 23 degrees C at day 0, monitored for a baseline 24 hour day (day1) and were shifted to 29 degrees C in either the entire 12 hour day period (Light Period:LP) or 12 hour night period (Dark Period:DP) in day 2 and the condition was repeated on day 3. Comparisons for change in sleep behavior is compared from day 1 (baseline) to day 2 and day 3 within the given manipulation and observed period.

Statistics

Data was analyzed using Prism Software (GraphPad). One-Way ANOVA analysis with a Bonferroni Multiple Comparison Correction was used to determine differences between experimental genotypes (GAL4/DopR) and two controls: (GAL4/+) and (DopR/+) for all measures of sleep and activity. Identical comparative measures were employed for RNAi and TrpA1 experiments.

Immunohistochemistry

Adult Brains were dissected in 1xPBS containing 0.05% Triton-X 100 (PBT) and then fixed overnight at 4°C in 2% paraformaldehyde (EM Sciences) in PBT. They were washed 5x 10 min in 0.1% PBT, blocked 1 hr in 0.1% PBT with 0.5% BSA and 5% normal goat serum, and then incubated with primary antibodies overnight at 4°C. They were then washed, blocked, and incubated with secondary antibodies overnight at 4°C. Washed tissues were mounted on glass slides in Vectashield (Vector Laboratories), small pieces of broken coverslips served as posts, covered with a coverslip and sealed. Primary antibodies were rabbit anti-DopR (1:1,250, Kong et al. 2010), mouse anti-CD2 (1:50, Pierce), and mouse anti-Cherry (1:200, Biorbyt) and secondaries were goat anti-rabbit Alexa Fluor 488 and goat anti-mouse Alexa Fluor 594 (Life Technologies). The tissues were imaged on a Nikon Eclipse Ti C1 Confocal System using 1 um steps and 40x or 60x oil-immersion lens.

Results and Discussion

Given the broad innervation of dopaminergic neurons in the central brain and the potential for many different areas of complementary DopR regulation in the brain, we sought to both test new regions not associated with DopR function as well as regions previously characterized for DopR functions. We screened for rescue of the increased sleep of *DopR/+* mutants. Table 1 summarizes our screen results for FlylightGal4 and Kaiser Gal4 lines associated with different regions of the *Drosophila* brain. The majority of lines screened failed to show significant differences from controls for sleep duration, used as primary criteria to characterize robust rescue and full restoration of DopR function. Although the ellipsoid body and noduli of the

central complex express high levels of DopR protein (Kong *et al.*, 2010, Lebestky *et al.*, 2009), these structures do not appear to be related to DopR function in sleep. Additionally, DopR is normally expressed in the optic lobe (OL), and restoration does not appear to modulate the sleep phenotype. Similar to known results from the Kume lab (Ueno *et al.*, 2012) that show the dorsal fan-shaped body (FSB) influences dopaminergic regulation of sleep, the c5-Gal4 line displays rescue of sleep phenotypes (Table 1). However it should also be noted that this Gal4 line also expresses strongly in the mushroom body. Given the known relationship of the FSB to sleep regulation, we sought to pursue new regions in the brain that could influence dopaminergic regulation of sleep, such as the antennal lobe, sub-esophageal ganglion, and AMMC. However, these and other specific regions tested do not appear to influence sleep phenotypes (Table1).

The mushroom body displays a non-uniform response when assessing DopR function. A number of Kaiser Collection Gal4 lines (201y, 117y, 43y, 30y), that have been used by the *Drosophila* community for manipulation of mushroom body properties failed to display significant phenotypes when restoring DopR function. It should be noted that for two of these lines, the Gal4/+ control showed low activity/high sleep patterns that could obscure a clear shift in restoration of sleep behavior (data not shown). However, rescue was observed for c305a, c5, and four Flylight Gal4 lines that express in the mushroom body: R23D03, R87E08, R87B01, H05 (Figures 2 & 3).

The Janelia Fly Light Collection is composed of Gal4 lines containing evolutionarily conserved transcriptional enhancer sequences derived from genes

expressed in the adult *Drosophila* brain. Therefore, we also tested Gal4 enhancer lines that were specifically derived from the regulatory enhancer sequences of the DopR locus (CG9652), summarized in Table 2. Of the eight lines tested, two lines R72B02 and R72B08 displayed significant rescue of the DopR sleep phenotype (Figure 2D-F). These lines also express significantly in the mushroom body, and the R72B08/DopR rescue condition not only restores daytime sleep, but also nighttime sleep to wildtype levels. It should also be noted that two of the non-rescue lines tested also display expression in the mushroom body. Whether this negative data reflects low or insufficient Gal4 expression in the rescue condition, failure of overlapping expression with endogenous DopR circuits in the mushroom body, or potentially opposing roles for DopR dependent sleep regulation in subsets of the mushroom body and/or other Gal4-expressing regions within the given pattern is unknown. Independent of the observed relationship between DopR and the mushroom body as "pro-wake", these experiments also potentially identified an opposing role for DopR as a "pro-sleep" modulator in the pars intercerebralis. When expressing DopR in the rescue condition, using the R72B03 Gal4 line, excessive sleep or inactivity is induced (Figure 2F).

In reviewing the lines tested, a common spatial determinant arising from our screening data that had the greatest effect on daytime sleep behavior appears to be the mushroom body (Figures 2-3). The lines R87E08, R87B01, R59H05 all restore daytime sleep, suggesting a new role for DopR in subsets of the mushroom body neurons (Figure 3). In all cases, the daytime sleep is restored, yet nighttime sleep is unaffected by DopR manipulation (Figure 3D-F). Furthermore, a clear separation of

DopR function in day-time sleep parameters versus night-time sleep parameters is also observed by close secondary parametric characterization of these spatially restricted lines (File S1).

The Gal4 lines R87E08, R87B01, R59H05 all display exceptional regional specificity (Figure 4). All three Gal4 lines show strong, restricted expression to subsets of neurons projecting within the alpha/beta lobes of the mushroom body (Figure 4 A-C). In all cases, we observe overlap of DopR protein expression (green) and Gal4-driven CD2-mCherry within the mushroom body (magenta)(Figure 4 A-C). The R87B01 Gal4 line projects solely within alpha/beta tracts of the mushroom body (Figure 4 A, D, E), whereas R59H05 and R87E08 also show sparse expression in the mushroom body gamma lobes (Figure 4B, C).

To confirm the requirement for DopR1 in the neuronal subsets of the mushroom body, we drove expression of an inducible UAS-DopR1-RNAi line (Keleman *et al.*, 2012) in these cells and assessed effects on sleep (Figure 5). Both lines tested, R87B01 and R87E08, show increased daytime sleep, consistent with a specific requirement for DopR in the MB β/α lobes for daytime sleep regulation. As proof of principle, these experiments suggest that the screening methodology used with the *DopR*^{f02676} allele is capable of positively identifying DopR^{+} neural circuits that influence sleep parameters.

Mushroom body Gal4-lines positively identified from the screen were also characterized by driving expression of UAS-TrpA1, to conditionally increase neuronal activity when elevating the environmental temperature (Figures 6-7). Neuronal activation of R59H05 during the night period displays a significant

positive shift in activity and loss of sleep (Figure 6 A, C, E). However, activation of these neurons during daytime increases sleep during the day (Figure 6 B, D, F). These phenotypes are different from the activation of R87E08 neurons, which show no phenotypic effect during night-time activation (Figure 7 A, C, E), but do show loss of sleep when activating the neurons during the day (Figure 7 B, D, F). These data suggest a role for these neuronal subsets in regulation of sleep behavior, and R87E08 has a role consistent with the predicted relationship for DopR function as "pro-wake" during the day period. R59H05-Gal4 expressing neurons show a paradoxical "pro-sleep" behavior when activated during the daytime, in that peak dawn and pre-dusk activities are elevated, but the "trough" of mid-day sleep is robust and an overall increase in sleep is observed (Figure 6B). This may suggest a more complicated modulatory relationship with dopamine in the phases of sleep regulation during the day, and the presence of additional molecular regulators in the overall function of these particular neurons.

Previous data suggested that DopR function in the whole mushroom body was not involved in sleep regulation, whereas the dorsal fan-shaped body (dFSB) plays a primary role (Ueno *et al.*, 2012). The data was based on genetic restoration of DopR function using the OK107 Gal4 line that expresses broadly in the mushroom body. One source of difference is the number of lines in the mushroom body that were tested in our study. Additionally, OK107 expresses in multiple regions outside of the mushroom body, including the median bundle/pars intercerebralis, tritocerebrum, sub-esophageal ganglion, antennal lobe, optic lobe, lobula, medulla, and transmedullary neurons (Morante & Desplan, 2008). Additionally our results

for R72B03 that increased sleep levels due to expression of DopR in the median bundle/pars intercerebralis (Figure 2F), suggest a possibility that two opposing functions (pro-wake and pro-sleep) within OK107 may have partially obscured the role of DopR in the mushroom body. In contrast, the R87E08, R87B01, R59H05 Gal4 lines show increased specificity within the mushroom body, and also mark a subset of the total MB neurons. Similar to recent studies that argue for microcircuit functions that individuate functions of a larger structure (Seidner *et al.*, 2015, Sitaraman *et al.*, 2015a), our data suggests that subsets of neurons in the mushroom body may mediate "pro-wake" regulation of daytime sleep.

Recent circuit activation experiments, utilizing UAS-TrpA1, have shown wake promoting roles for the dopaminergic PPL1 and PAM subpopulations of neurons that innervate subsets of the α/β, α'/β', and γ mushroom body (Sitaraman *et al.*, 2015b). Our data suggests a complementary genetic requirement for DopR in subsets of mushroom body function. We do acknowledge the possibility that small contributions due to Gal4 expression outside of the mushroom body for any given line may contribute or act combinatorially with MB-Gal4 expression to influence sleep phenotypes. Thus, it will be informative to look at precise neural subsets within our positive circuit hits, using MARCM clones or Split-Gal4 reagents to further subdivide roles for our neuronal populations and potentially identify minimal sufficient neurons required for the phenotypes observed. Regardless of whether regions act combinatorially or individually, our study has operationally identified multiple new DopR^{+} neuronal subpopulations that appear to influence sleep in *Drosophila.* It will also be useful to further investigate a potential

requirement for DopR function in the gamma lobes, as opposed to other Dopamine receptor homologs that may be functioning in that structure. The data for R87E08-Gal4, and R59H05-Gal4, (Figures 3 & 4) may support a potential role for *DopR* in the gamma lobe in "pro-wake" behavior during the day.

Our data collectively suggest that DopR in anatomical subsets of the mushroom body regulates daytime sleep with no significant impact on DopR-regulated night sleep patterns (Figure 3). This supports an interpretation of potential circuit separation of DopR-expressing neurons that regulate day versus night sleep and arousal patterns, consistent with a large role for the dorsal Fan-shaped body in night sleep regulation (Ueno *et al.*, 2012). Furthermore, distinctly different levels of cocaine-induced activity observed for wildtype animals during the day (low induced activity) versus night (high induced activity) suggests potential separability between day and night that is nevertheless dependent in part on DopR function and may support spatially distinct circuits (Lebestky *et al.* 2009). Given the separability of day and night circadian oscillators (Grima *et al.*, 2004, Stoleru *et al.*, 2004) and the differential expression of arousal activities in the day and night (Ishimoto *et al.*, 2012, Parisky *et al.*, 2016), this data identifies a requirement for DopR in the mushroom body for daytime sleep and arousal regulation. Future immunohistochemical and double mutant analyses with *DopR* and previously identified day sleep modulators, such as sex peptide (Isaac *et al.*, 2010), Ecdysone receptor and DTS-3 (Ishimoto *et al.*, 2012), may also be informative in better understanding the network of molecules involved in day sleep regulation.

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Figure Legends

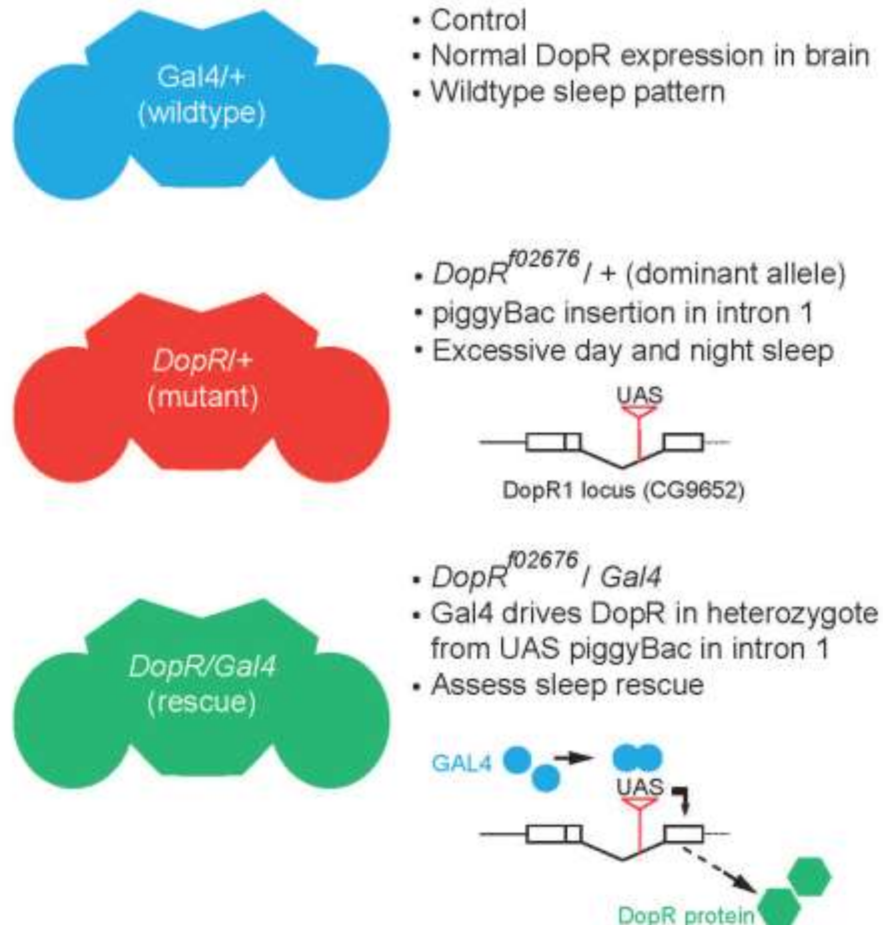

Figure 1: Schematic representation of screen conditions. Restoration of normal wildtype sleep and activity patterns is assessed relative to selective restoration of DopR function in subsets of neurons in the *drosophila* adult brain. If sleep levels in the rescue genotype (*Gal4/DopR*^{f02676}) are equivalent to wildtype behavior (*Gal4/+*) and significantly different from *DopR*^{f02676}/+ as measured by one-way ANOVA comparison, the neuronal Gal4 line is regarded as a successful rescue.

Table 1

Janelia Flylight Gal4	Genomic Origin	Site(s) of Primary Expression	Sleep Rescue
R14HO4	CG9907	AL	Fail
R70E03	CG30361	AL	Fail
R73D06	CG9097	NOD	Fail
R83H12	CG1849	NOD	Fail
R53B06	CG16766	LH	Fail
R10B01	CG7664	OL	Fail
R17F12	CG3454	OL	Fail
R33H10	CG7524	OL	Fail
R20D05	CG9554	CAN	Fail
R21H12	CG5610	CAN	Fail
R27E02	CG32171	AMMC	Fail
R64C04	CG7395	AMMC	Fail
R83C03	CG1849	AMMC	Fail
R23C12	CG14307	PL PROTOCER	Fail
R22H09	CG2872	SOG	Fail
R26G08	CG10772	SOG	Fail
R27G01	CG32171	SOG	Fail
R32B04	CG10388	SOG & OL	Fail
R45H03	CG1429	AMMC & AL & MB	Fail
R59C12	CG31665	OL & AMMC & MB & EB	Fail
R59G03	CG7958	MB & FSB & OL	Fail
R75F05	CG4807	MB & PCB & LH	Fail
R37G12	CG1004	FSB & LH & PCB	Fail
R82G02	CG1133	MB & BULB & PCB	Fail
R21D08	CG6383	MB SUBSET & LH & PROW	Fail
R59H07	CG32296	MB SUBSET	Fail
R23D03	CG14307	MB & PCB & LH & SOG	Rescue
R87E08	CG3340	MB SUBSET	Rescue
R87B01	CG18389	MB SUBSET	Rescue
R59H05	CG7467	MB SUBSET	Rescue
Kaiser Collection	Genomic Origin	Site(s) of Primary Expression	Sleep Rescue
c119	unknown	EB	Fail
36y	unknown	EB	Fail
95y	unknown	EB	Fail
c232	unknown	EB	Fail
201y	unknown	MB	Fail
117y	unknown	gamma MB	Fail
43y	unknown	MB	Fail
30y	unknown	MB & FSB & EB & OL & PI & LH	Fail
c305a	unknown	MB	Rescue
c5	unknown	dFSB & MB	Rescue

Table 1: Neural circuits screened and expression patterns in adult *drosophila* brain. Sites of Primary Expression is operationally defined as neuroanatomical regions that display highest expression by review of confocal stacks from Fly-Light expression studies. Abbreviations for brain regions expressing Gal4: AL: Antennal

Lobe. NOD: Noduli. LH: Lateral Horn. OL: Optic Lobe. CAN: Cantle. AMMC: Antennal Mechanosensory and Motor Center. PL PROTOCER: Posterior Lateral Protocerebrum. SOG: Subesophageal Ganglion. MB: Mushroom Body. FSB: Fan Shaped Body. EB: Ellipsoid Body. PCB: Protocerebral Bridge. PI: Pars Intercerebralis/Median Bundle.

Figure 2

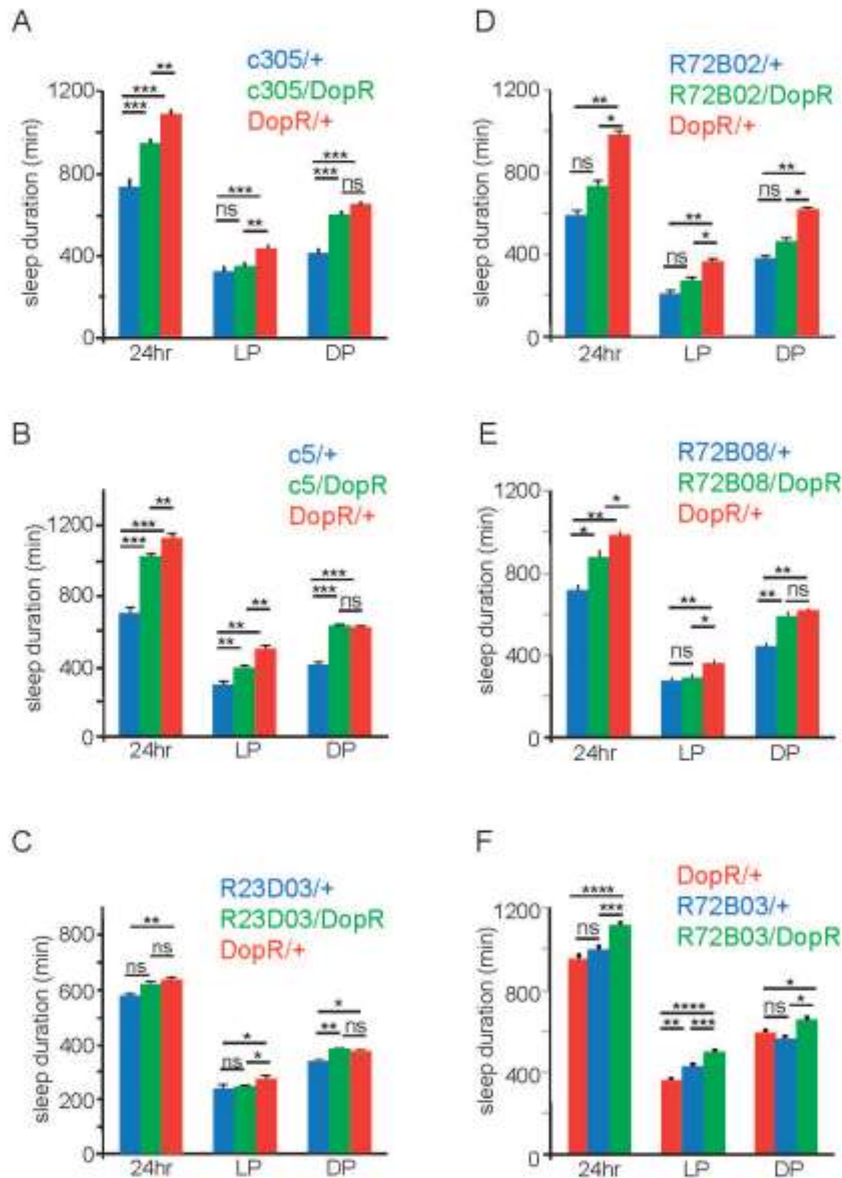

Figure 2: Rescue of Day Sleep by Broad Gal4 Expression including the Mushroom Body. A-F) Genotype comparison of sleep duration for rescue (Blue: Gal4/+)(Green: Gal4/DopR)(Red: DopR/+). Sleep duration measured in minutes for either 24 hour, light period (LP), and dark period (DP). A) c305 Gal4 comparison. B) c5 Gal4 comparison. C) R23D03 Gal4 comparison. D-F) Rescue of Day Sleep by Gal4 lines

derived from DopR genomic enhancer elements (CG9652). D) R72B02 Gal4 comparison. E) R72B08 Gal4 comparison. F) R72B03 Gal4 comparison. n=32 for all conditions. Statistics by ANOVA and bonferroni correction: * $p < .05$, ** $p < .01$, *** $p < .001$, **** $p < .0001$. ns = non-significant.

Table 2

Janelia Flylight Gal4	Genomic Origin	Site(s) of Primary Expression	Sleep Rescue
R72B09	CG9652	EB & LH & SOG & MB	Fail
R72B12	CG9652	MB & AL & FSB & NOD & BROAD LOW	Fail
R72C02	CG9652	MB & OL & AL & LH & BROAD LOW	Fail
R72B10	CG9652	AMMC & LH & PCB & SOG	Fail
R72C01	CG9652	AMMC & FSB & PCB & OL & SOG & MB	Fail
R72B03	CG9652	PI & PROW & AL & ANTLER	Increased Sleep
R72B02	CG9652	MB & OL	Rescue
R72B08	CG9652	MB & OL & AL	Rescue

Table 2: Screened Gal4 lines derived from DopR genomic enhancer elements (CG9652). Sites of Primary Expression is operationally defined as neuroanatomical regions that display highest expression by review of confocal stacks from Fly-Light expression studies. Abbreviations for brain regions expressing Gal4: AL: Antennal Lobe. NOD: Noduli. LH: Lateral Horn. OL: Optic Lobe. CAN: Cantle. AMMC: Antennal Mechanosensory and Motor Center. PL PROTOCER: Posterior Lateral Protocerebrum. SOG: Subesophageal Ganglion. MB: Mushroom Body. FSB: Fan Shaped Body. EB: Ellipsoid Body. PCB: Protocerebral Bridge. PI: Pars Intercerebralis/Median Bundle.

Figure 3

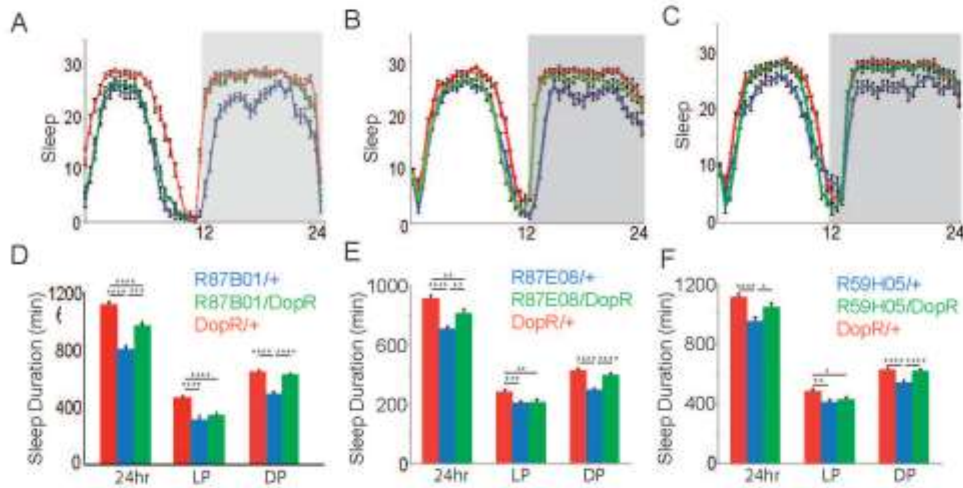

Figure 3: Rescue of Day Sleep by Gal4 Expression in subsets of the Mushroom Body. A-F) Genotype comparison of sleep and wake duration for rescue (Blue: Gal4/+)(Green: Gal4/DopR)(Red: DopR/+). A-C) Sleep in 30 minute bins represented for all genotype conditions over 24 hour period. D-F) Sleep duration measured in minutes for either 24 hour, light period (LP), and dark period (DP). A, D) R87B01 Gal4 comparison. B, E) R87E08 Gal4 comparison. C, F) R59H05 Gal4 comparison. n=32 for all conditions. Statistics by ANOVA and bonferroni correction: * $p < .05$, ** $p < .01$, *** $p < .001$, **** $p < .0001$.

Figure 4

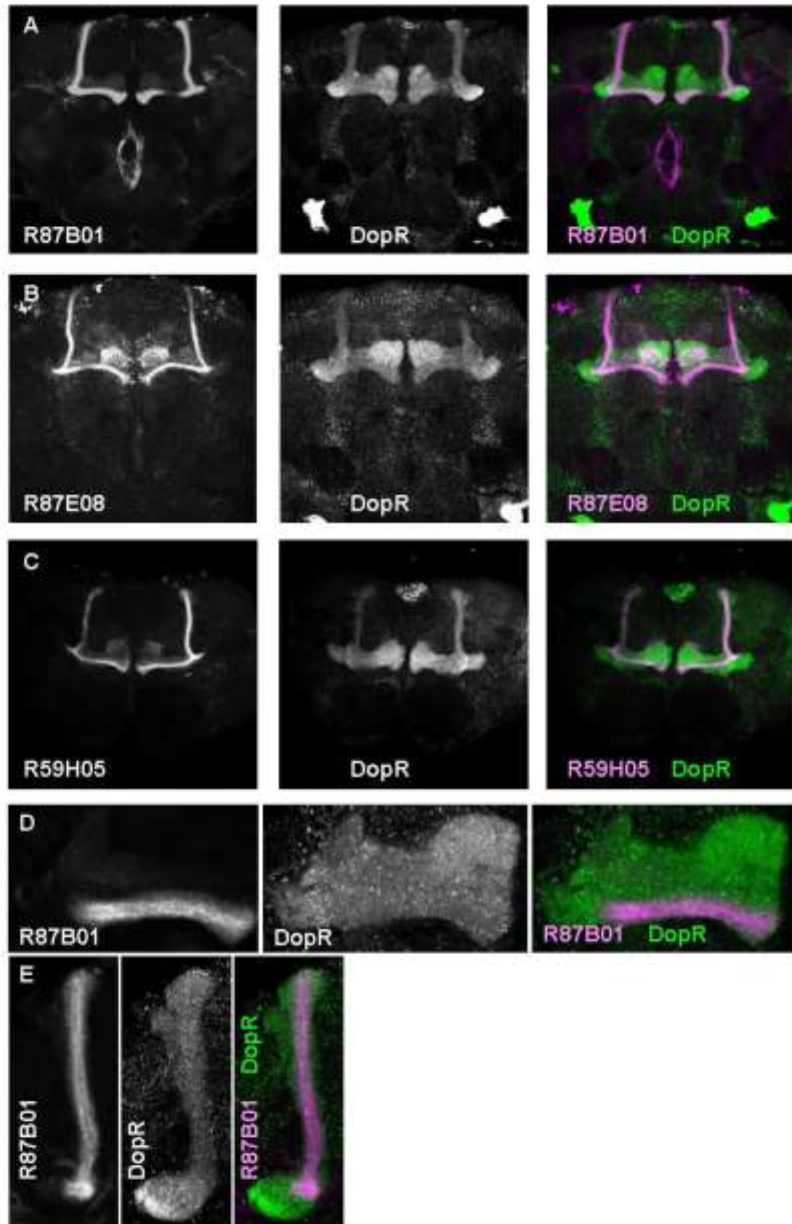

Figure 4: GAL4 and DopR expression patterns in the adult brain. Compressed confocal z-stacks of brains expressing UAS-CD2mCherry (magenta) in the indicated Gal4 patterns and immunostained for the DopR protein (green). A) R87B01 - 30um z-stack. B) R87E08 - 25um z-stack. C) R59H05 - 20um z-stack. D, E) Higher magnification images of the beta/gamma (D) and alpha (E) lobes of the mushroom bodies of R87B01>CD2mCherry brains. D) 3um z-stack. E) 10um z-stack.

Figure 5

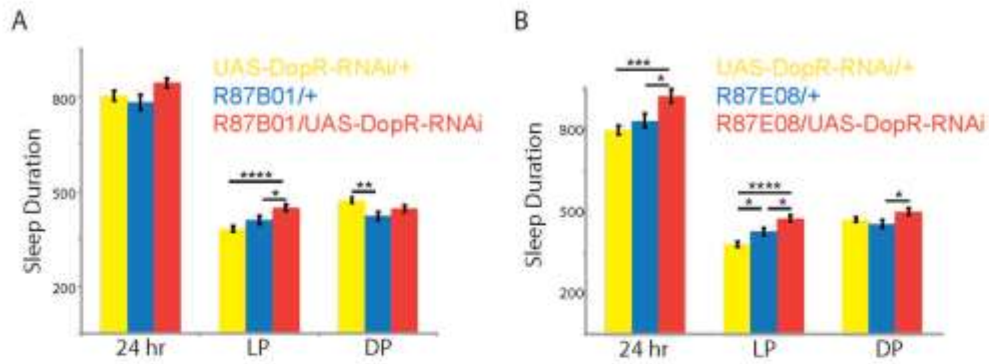

Figure 5: RNAi Knockdown of DopR expression in subsets of mushroom body neurons. A, B) Genotype comparison of sleep duration (Yellow: UAS-DopR-RNAi) (Blue: Gal4/+)(Red: DopR/+). Sleep duration measured in minutes for either 24 hour, light period (LP), and dark period (DP). A) R87B01 Gal4 comparison. B) R87E08 Gal4 comparison. n=32 for all conditions. Statistics by ANOVA and bonferroni correction: * p < .05 , ** p < .01, *** p < .001, **** p < .0001.

Figure 6

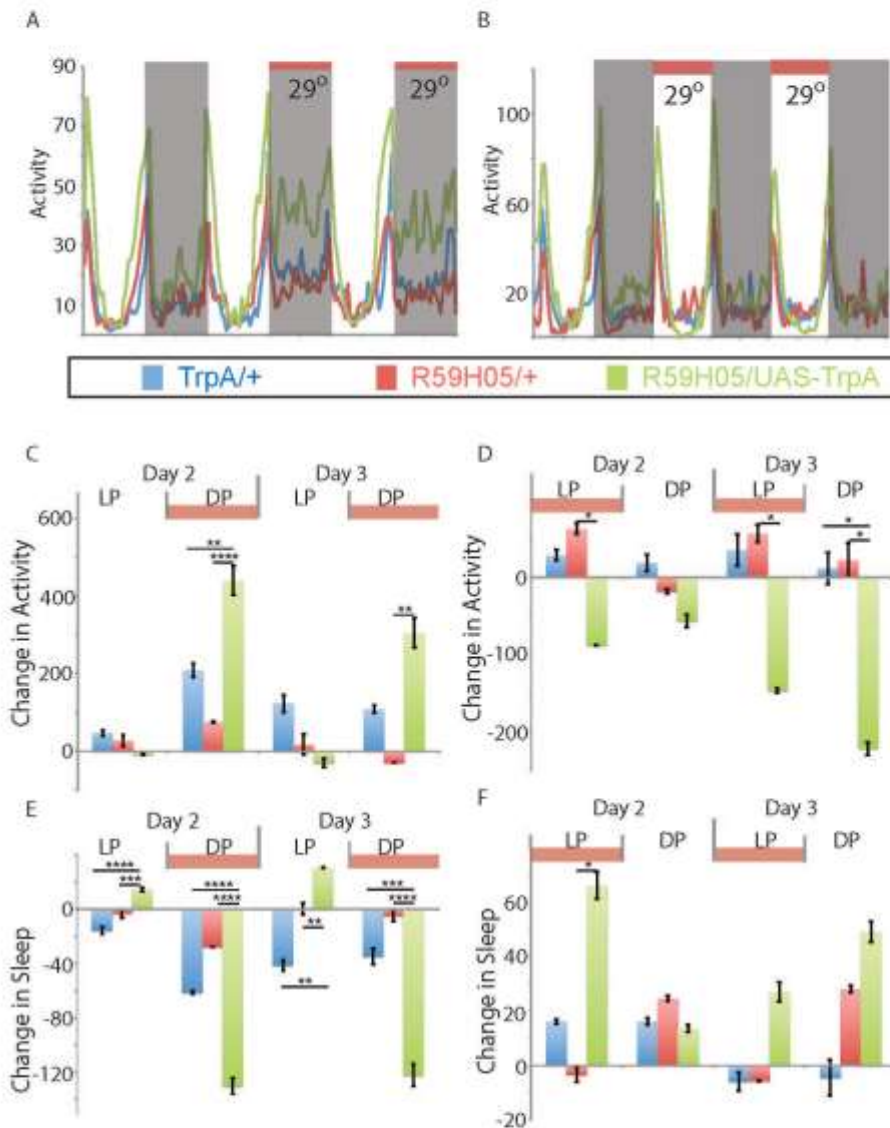

Figure 6: TrpA1 activation of R59H05-Gal4 expressing neurons. A-F) UAS-TrpA1/+ (blue), R59H05/+ (red), and R59H05/ UAS-TrpA1 (green). A, B) Activity plots for baseline (23°) and induced conditions (29°). C-G) Change in activity (C, D)or sleep (E, F) on Day 2 and 3 as compared to Day 1 measured in minutes for either 24 hour, light period (LP), and dark period (DP). n=28-32. Statistics by ANOVA and bonferroni correction: * $p < .05$, ** $p < .01$, *** $p < .001$, **** $p < .0001$.

Figure 7

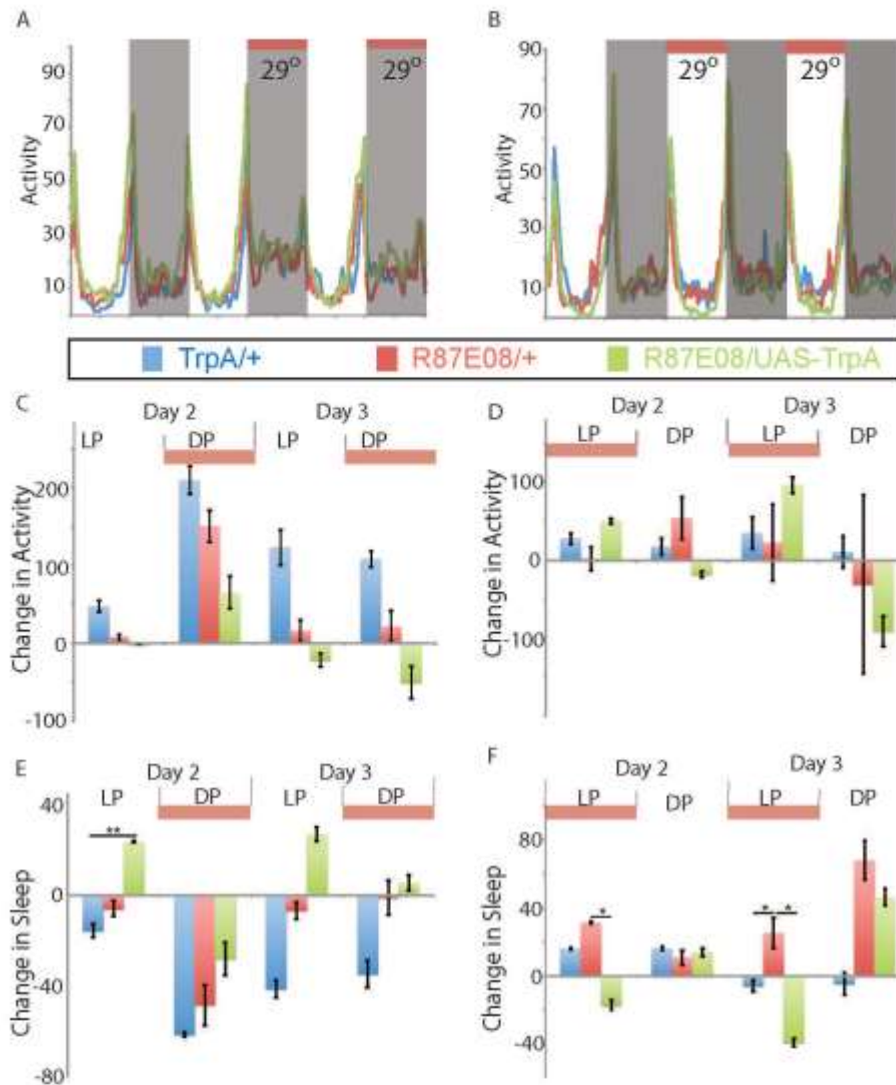

Figure 7: TrpA1 activation of R87E08-Gal4 expressing neurons. A-F) UAS-TrpA1/+ (blue), R87E08/+ (red), and R87E08/ UAS-TrpA1 (green). A, B) Activity plots for baseline (23°) and induced conditions (29°). C-G) Change in activity (C, D)or sleep (E, F) on Day 2 and 3 as compared to Day 1 measured in minutes for either 24 hour, light period (LP), and dark period (DP). n=28-32. Statistics by ANOVA and bonferroni correction: * $p < .05$, ** $p < .01$, *** $p < .001$, **** $p < .0001$.