A day sleep promoting role of phototransduction in *Drosophila melanogaster*

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Abstract:

The daily sleep-wake cycle is a conserved behaviour defined by locomotion quiescence and enhanced responsive threshold to sensory stimuli. Both the circadian clock and sleep-homeostasis determine the daily sleep profile. Environmental light is a major sensory input and also regulates circadian clock and the balance between sleep and wakefulness. In *Drosophila*, the cellular mechanism and neural circuitry underlying light-mediated circadian synchronization are well-established, yet the direct relationship between light/visual input and sleep remains unclear. To address this knowledge gap, we measured sleep behaviour in *Drosophila* with mutations in genes involved in phototransduction and downstream neural transmission. We observed consistent day sleep fragmentation in flies with mutations in multiple phototransduction components. We also found that mutation that led to hyperpolarised *Drosophila* photoreceptors resulted in shorter day sleep. We found a severe reduction in locomotor speed in several visual mutants during normal waking time preventing assessment of their sleep-linked immobility. Taken together, our rigorous quantification of sleep in phototransduction genetic mutants reveals the key role of visual input in promoting sleep.

1. Introduction

Sleep is vital for good health. Disruptions to either sleep timing and/or amount has been linked to numerous metabolic and neurological diseases (see reviews, Hartmann and Kempf, 2023; Wulff et al., 2010). Across the animal kingdom, sleep is defined by behavioural quiescence and increased arousal threshold to environmental stimuli such as light or mechanic vibrations. Arousal threshold within a period of sleep varies in stages that are associated with specific electrophysiological signatures in electroencephalogram (EEG) and local field potentials (LFPs) in the brain (Lakhiani et al., 2023). With its versatile genetic toolkit and conserved behavioural and electrophysiological features of sleep (Jagannathan et al., 2024; Shafer and Keene, 2021), the fruit fly *Drosophila melanogaster* has been used for identifying the biological basis and the conserved function of sleep. In parallel to murine investigations, studies in *Drosophila* have demonstrated that neuronal redox status may form a cellular basis of sleep (Hartmann and Kempf, 2023) and sleep stages play roles in synaptic scaling that is important for learning and memory (Dissel et al., 2015; Gilestro et al., 2009; Tainton-Heap et al., 2021; Yap et al., 2017). Recent investigations in *Drosophila* have also shown that sleep is required for effective maintenance of the immune system, gut microbiome, and waste clearance (Alphen et al., 2021; Vaccaro et al., 2020; Vincent et al., 2022).

Daily sleep onset and duration are regulated by two intrinsic mechanisms: the circadian clock and sleep homeostasis. The circadian clock defines the baseline sleep-wake level across 24 hours, whereas sleep homeostasis maintains the overall level of sleep amount. In *Drosophila*, the neural circuits that generate circadian rhythm are composed of 242 clock neurons (Reinhard et al., 2024) that express the core molecular clock mechanism. Whereas the homeostatic control of sleep is distributed across the following neural domains: Pars Intercerebralis (PI), Mushroom Body (MB), Fan-Shaped Body (FSB) and Ellipsoid Body (EB) (Jones et al., 2025; Shafer and Keene, 2021). The circadian clock and sleep homeostasis are likely coordinated, as clock neurons modulate the FSB-mediated sleep homeostat (Hsu et al., 2025).

Light is a prominent environmental stimulus that regulates both circadian clock and sleep homeostasis to maintain an animal's temporal niche and sufficient sleep. The synchronisation between environmental light regime and the neural activity of clock neurons is called circadian photoentrainment. Visual inputs are part of photoentrainment in *Drosophila*. The visual system in the fly is composed of peripheral light sensing neurons: photoreceptors in compound eyes, HB-eyelet and ocelli. Photoreceptors in the eye are subdivided into outer achromatic R1-R6 and inner chromatic R7/R8 neurons, both of which sends their synaptic outputs to downstream visual processing brain areas called the optic lobes and ventrolateral neuropils (Nériec and Desplan, 2016).

58 Crucially, light signals are converted into depolarisation of the neuronal membrane via a cellular
59 process called phototransduction in the photoreceptors of the compound eye and HB-eyelet.
60 Phototransduction requires the light-sensing GPCR Rhodopsins 1-6 (Rh1-6) and their downstream
61 signal cascades that ultimately activate cation channels depolarising the photoreceptors (Hardie,
62 2012). Activation of photoreceptors results in synaptic release of histamine or acetylcholine to
63 communicate with clock neurons either directly or indirectly via a neural network in the optic lobes
64 (Alejevski et al., 2019; Ogueta et al., 2020, 2018; Rieger et al., 2003; Schlichting et al., 2016; Tang et
65 al., 2022; Xiao et al., 2023). Apart from via the visual system, *Drosophila* circadian clocks are
66 entrained by cell autonomous light-sensing mechanisms mediated by *cryptochrome* (*cry*), *quasimodo*
67 (*qsm*) or *Rhodopsin 7* (*Rh7*) (Chen et al., 2011; Ni et al., 2017; Peschel et al., 2009).

68 Light can directly regulate sleep by either promoting or supressing sleep via different neural
69 pathways in the murine system (Altimus et al., 2008; Pilorz et al., 2016). As a crepuscular species,
70 *Drosophila* sleep both in the day (light) and in the night (darkness). While current evidence indicates
71 that distinct neurogenetic networks may control day and night sleep (Chen et al., 2019; Damulewicz
72 et al., 2020; Hsu et al., 2025; Ishimoto et al., 2012; Lamaze et al., 2018; Mazzotta et al., 2020;
73 Sengupta et al., 2019), the mechanism underlying light-mediated regulation of the *Drosophila* sleep
74 homeostat is far from clear. Previous studies indicate that midday sleep (i.e., siesta during six hours
75 of the midday) can be enhanced by high-intensity light via the HB-eyelet-mediated cholinergic
76 pathway (Rieger et al., 2007; Schlichting et al., 2019). Separate studies however, have shown
77 contradictory findings for the role of compound eye photoreceptors in sleep regulation: synaptic
78 inhibition in these photoreceptors promote sleep (Damulewicz et al., 2020), yet the optogenetic
79 activation of the same photoreceptors in another investigation did not result in sleep suppression
80 (Kirszenblat et al., 2019). Moreover, artificial activation of the direct downstream target of eye
81 photoreceptors, lamina monopolar neuron 2 (L2 LMC), resulted in drastic sleep loss (Muraro and
82 Ceriani, 2015). This finding contradicts the increased sleep observed in flies with reduced histamine
83 (Oh et al., 2013), which is the main neurotransmitters released from photoreceptors to inhibit L2
84 LMC neurons (Pantazis et al., 2008). Furthermore, visual experience in light was recently found to
85 promote night sleep in *Drosophila* (Kirszenblat et al., 2019), suggesting light and/or vision *per se*
86 serve as sleep homeostat drivers, similar to in vertebrates (Horne and Walmsley, 1976; Korf et al.,
87 2017; Miyamoto et al., 2003).

88 A recent systematic study of mutants with defects in various light input pathways indicate that only
89 *Rh7* is required for shade sleep preference in the day, but the study did not investigate the baseline
90 sleep level in these mutants (Meyerhof et al., 2024). We therefore investigated the role of light input
91 pathways in *Drosophila* sleep by focusing on the role of the visual system. Using the conventional

92 infrared beam-based sleep assay (Chen et al., 2019; Chiu et al., 2010), we surveyed baseline sleep
93 profiles in three groups of flies: strains with mutations in genes involved in (i) phototransduction
94 and (ii) downstream histaminergic neurotransmission (Hardie, 2012), and (iii) those with artificial
95 inhibition of photoreceptors. We also performed electroretinogram (ERG) examine the
96 phototransduction/neurotransmission defects in these above mutants. By carefully examining sleep
97 and ERG parameters, we identified a novel role of visual phototransduction in driving sleep.

98 **2. Materials and methods**

99 **2.1 Fly husbandry**

100 *Drosophila* strains were kept in standard fly food (glucose 6.93% w/v, agar 0.5% w/v, cornmeal, 6.3%
101 w/v, yeast, 4.38% w/v, propionic acid, 0.26% v/v, 0.2% v/v nipagin) at 25°C 12-hour light dark cycle.
102 Long-term fly stocks are kept in the same food and light regime at 18°C. The Canton S and Oregon R
103 wildtype strains were obtained from the Kyriacou Lab (University of Leicester, UK), while w^{iso31} (i.e.
104 *iso*) control was obtained from the Jepson lab (University College London, UK). The following visual
105 mutants, Gal4 and UAS transgenic flies were ordered from Bloomington Drosophila Stock Center
106 (Indiana University, USA) (stock number): trp^{1} (5692); trp^{MB} (23636); $trpl^{302}$ (31433); $trpl^{MB}$ (29134);
107 $ninaE^{8}$ (2001); $ninaE^{5}$ (3531); $ninaE^{17}$ (5701); $norpA^{P24}$ (9048); hdc^{MB} (25260); hdc^{JK910} (64203); ort^{1}
108 (1133); ort^{attP} (84559); *Rh1-Gal4* (8691); *gmr-Gal4* (1104); *UAS-kir2* (6595); *UAS-TeTxLC* (28837);
109 *UAS-myr-mRFP* (7118). All Gal4 and UAS transgenic flies were brought into the w^{iso31} background via
110 five generation outcrossing following *[w+]* eye colour marker cassette. For *Rh1-Gal4* outcrossing, we
111 generated *UAS-myr-mRFP/+; Rh1-Gal4/+* flies which expresses RFP in the eyes, we then crossed this
112 line to w^{iso31} and used this RFP signal as a visible marker under a fluorescent stereoscope to ensure
113 outcrossing offspring still contained both *UAS-myr-mRFP* and *Rh1-Gal4* transgenes. The final stock
114 was then established by balancer crossing with w^{iso31C}*;Sco/CyO; MKRS/TM6B* and then w^{iso31}*;+/+;*
115 *MKRS/TM6B* for removing *UAS-myr-mRFP*. Among other lines, hdc^{MB}, ort^{attP}, trp^{MB}, and $trpl^{MB}$
116 contained either GFP or RFP visible markers, but the rest are classic missense mutations, making
117 outcrossing without laborious PCR-based sequencing difficult. For simplicity, sleep experiments for
118 all non-Gal4/UAS mutants were conducted without outcrossing and the only consistent results
119 among mutant alleles of each gene were interpreted. Following the unexpected but consistent night
120 sleep among *hdc* mutants, we decided to minimise the genetic background difference among these
121 lines: since the precise genetic lesion of hdc^{JK910} on the *hdc* loci at the 2nd chromosome is unknown,
122 only the 1st and 3rd chromosomes of hdc^{JK910} were replaced with those from w^{iso31} stock via the
123 following successive crossings with balancer lines (gifts from Jepson lab):

124 P: w^{iso31}/w^{iso31}*; Sco/CyO; MKRS/TM6B* x *+/Y;* hdc^{JK910}/hdc^{JK910}*; +/+*

125 F1: w^{iso31}/w^{iso31}; *Sco/CyO*; $+^{iso31}/+^{iso31}$. x w^{iso31}/Y; hdc^{JK910}/CyO; *TM6B/+*

126 F2: w^{iso31}/w^{iso31}; hdc^{JK910}/CyO; *TM6B*/$+^{iso31}$. x w^{iso31}/Y; hdc^{JK910}/CyO; *TM6B*/$+^{iso31}$

127 F3: w^{iso31}/w^{iso31}; hdc^{JK910}/CyO; $+^{iso31}/+^{iso31}$. x w^{iso31}/Y; hdc^{JK910}/CyO; $+^{iso31}/+^{iso31}$

128 Separately, hdc^{MB} flies were brought into the w^{iso31} background via five generation outcrossing
129 following the *GFP* eye marker.

130 **2.2 Sleep recording and analysis**

131 *Drosophila* sleep, particularly day time sleep, is highly dependent on by sex, mating and nutrient
132 status (Dove et al., 2017; Duhart et al., 2023; Isaac et al., 2009; Wu et al., 2018). Typically, mated
133 females can sleep for as little as 150-200 minutes compared to the 300-400 minutes shown by males
134 (out of the total 720 minutes of day time). To capture both sleep increase and decrease upon genetic
135 manipulation, we focused our investigation on virgin male flies. Moreover, by focusing on males, our
136 data is comparable to the previous decades of photoentrainment investigations of *Drosophila*
137 circadian research that has been conducted mainly on male visual mutants (e.g. Rieger et al., 2003
138 and see review in Mazzotta et al., 2020). As such, we would caution against extending our findings to
139 those of sleep in female flies.

140 Prior to the sleep experiment, *Drosophila* strains or crossings were reared in food with a richer
141 recipe (wheat germ 1% w/v, soy 0.8% w/v, glucose/sucrose 3.3%/1.5% w/v, treacle 3% v/v, agar 1%
142 w/v, cornmeal 1.5% w/v, yeast 3.5% w/v, propionic acid 0.5% v/v, nipagin 0.1% v/v), so as to keep
143 the rearing conditions consistent with previous experimental conditions (Chen et al., 2019, 2011;
144 Lamaze et al., 2018). We also note that in general, all strains of flies are less motile or less
145 reproductively successful in standard fly food (as described above in 2.1). Sleep recordings were
146 performed as described in (Chen et al., 2019). Three to four day-old virgin adult males were
147 transferred to typical behaviour tubes containing food (2% w/v agar and 4% w/v sucrose at one end
148 and cotton wool at the other), and housed in typical Drosophila Activity Monitor (DAM, Trikinetics
149 Inc.) for 3 days of 12hours light-dark cycle at 25°C in a temperature and light controlled incubator
150 (MIR-254-PE, PHCbi). The lighting source was a day light fluorescent lamp (FL15D, 6500K) with
151 intensity between 1.2 and 6.7 w/m^2 depending on the distance to the lamp. With conservative
152 conversion with luminance surface area between 0.25 to 1 m^2, our light intensity was approximately
153 equal to 402 to 1608 lux. The locomotion of each fly was recorded as number of infrared beam
154 crosses per minute. The locomotion data for day 3 was transferred to a previously customised Excel
155 Calculator (Chen et al., 2019) to calculate all sleep episodes (defined as any period of immobility ≥ 5
156 minutes) along 24 hours. Additionally, the following parameters were calculated for each fly: **overall**

157 **sleep profile**: minutes of sleep per 30 mins across 24 hours; **day sleep**: total minutes of sleep during
158 12 hours of day; **night sleep**: total minutes of sleep during 12 hours of night; **day average sleep bout**
159 **length**: day sleep minutes divided by total number of sleep bout during 12 hours of day; **night**
160 **average sleep bout length:** night sleep minutes divided by total number of sleep bout during 12
161 hours of night; **day waking activity**: number of beam crosses divided by total wake minutes during
162 12 hours of day**; night waking activity**: number of beam crosses divided by total wake minutes
163 during 12 hours of night.

164 **2.3 Electroretinogram (ERG)**

165 Three-to-five day-old adult virgin male flies were inserted into a p1000 plastic pipette tip, so that its
166 head protruded from the narrow ending. The head was fixed using melted honey wax applied
167 between the thorax and head without blocking the mouthpart and eyes. The pipette tip mounted fly
168 was kept in the dark for 20 mins and then transferred into the ERG recording rig. Glass electrodes
169 (Borosilicate) with resistance of ~5 MO and filled with 3 M KCl were inserted into the compound eye
170 (recording) and head (reference). Each fly was left in the dark for 3 minutes before being exposed to
171 four light intensities: 0.004, 0.02, 0.1 and 0.5 w/m^2 at 540nm LED green light source (M530L4-C1,
172 ThorLab). These intensities fall within the liner range of receptor potential response. We stimulated
173 with 1000 ms exposure to each light intensity and repeated the procedure two further times to
174 obtain an average value of three presentations at each light intensity for each fly. The potential
175 difference between the electrodes was amplified 10x, and acquired by Neuroprobe Amplifier model
176 1600 and 8-channel data acquisition interface DA-8 (A-M systems) using the SciWorks Data
177 Acquisition & Analysis Suite.

178 **2.4 Data display and statistics**

179 The `ggplot2` package (3.5.2) in R (4.4.2) used to display sleep and ERG data. X-Y plots were used to
180 display overall sleep profiles for each compared genotype. Boxplots with average values of each
181 individual were used to display sleep parameters data. We calculated the average voltage change of
182 on transient, receptor potential and off transient in the ERG profiles of each replicate for each fly
183 (Figure 1H). The value for each fly was then plotted against light intensity on logarithmic scales by
184 `ggplot2`. Considering the variability in sleep data (Chen et al., 2019; Lamaze et al., 2018), statistical
185 significance was identified by Kruskal-Wallis rank sum test for non-parametric multiple comparison
186 (`kruskal.test`). Pairwise Wilcoxon rank sum exact test with multiple comparison Benjamini-
187 Hochberg correction (`pairwise.wilcox.test`) was used to specifically identify difference
188 between pairwise genotypes. Unlike sleep data, ERG voltage changes data were pooled across four
189 intensities when identifying genotypical difference via Pairwise Wilcoxon rank sum exact test.

Significance level is set at adj.p<0.05. Effect size *r* is calculated by `wilcox_effsize` with magnitude categories: small; moderate and large assigned. Importantly, while Benjamini-Hochberg correction is required to correct the p-values, the effect size *r* does not change by multiple comparison, as it is defined by $Z/\sqrt{n}$. All statistic comparisons, p values, effect size and sample size are listed in Table1 to Table 6 for the corresponding Figure 1 to Figure 5.

195 **3. Result**

196 **3.1 Defects in phototransduction genes caused short day sleep bout**

197 To verify the role of visual phototransduction genes in sleep regulation, we tested eight mutant fly
198 strains. These strains are mutants of the following four genes: *Rhodopsin 1/ninaE*, *Phospholipase*
199 *C/norpA*, and the two cation channels *trp* and t*rpl*. These included both classic missense mutations,
200 and when available, more recently characterised mutations using P-element insertion. To analyse
201 the details of sleep-wake profiles under a typical day of 12 hours light-12 hours dark, we examined
202 three conventional parameters for both day and night period: total sleep, average sleep bout length
203 and waking activity.

204 **3.1.1 *ninaE***

205 Upon exposure to light, 11-cis 3-hydroxy-retinal, the chromophore in the Rhodopsin protein
206 undergoes photoisomerization, transforming Rhodopsin to Metarhodopsin which then triggers a
207 downstream GPCR signal cascade. Rhodopsin 1/ninaE protein is the main light-sensing GPCR in the
208 outer photoreceptors R1-R6 of the *Drosophila* compound eye. Reduction or loss of ninaE abolishes
209 or reduces photoisomerization and therefore blocks the light-induced photoreceptor potential and
210 transients that are typically measured for R1-R6 in ERG (Hardie, 2012).

211 Many visual tasks, such as navigation, phototaxis, optomotor and escape, require R1-R6 activity. We
212 therefore first tested the sleep profile of the class null allele $ninaE^{17}$ (O'Tousa et al., 1985). During
213 the day, $ninaE^{17}$ mutants show a lower midday sleep peak in the sleep profile (Figure 1Ai) but the
214 amount of total sleep is comparable to the white eye control, w^{iso31} (here after *iso*, Figure 1Di).
215 However, we found that the average sleep bout length in $ninaE^{17}$ is shorter than the control,
216 indicating sleep fragmentation (Figure 1Bi). During the night, we observed increased sleep amount
217 (Figure 1Gi) while the average sleep bout length is similar to the control (Figure 1Ei). Since sleep
218 measurements in *Drosophila* depend on locomotion, an increase in sleep can be falsely claimed due
219 to defects in locomotion. Therefore, "waking activity", i.e., locomotor activity per waking minutes,
220 was designed as a conventional measure to detangle such a compound scenario (Andretic and Shaw,
221 2005; Harbison and Sehgal, 2008). We applied this measurement and found that waking activity is
222 similar between $ninaE^{17}$ and the control in both day and night (Figure 1Ci,Fi).

223 $ninaE^{17}$ flies contain a secondary $ebony^{1}$ mutation that impacts sleep-wake cycles (Rossi et al., 2015;
224 Suh and Jackson, 2007). We therefore tested two other *ninaE* hypomorphs: $ninaE^{5}$ and $ninaE^{8}$
225 (Scavarda et al., 1983). Both mutants show similar midday sleep peak reductions to those seen in
226 $ninaE^{17}$ flies (Figure 1Aii-iii). This observation is consistent with the significant reduced day sleep

227 observed in either mutant when compared to the control (Figure 1Dii-iii). Similarly, both mutants
228 also show shorter average sleep bout length in the day (Figure 1Bii-iii). Intriguingly, the loss of sleep
229 in the two *ninaE* hypomorphs was observed despite of the loss of waking activity in the day (Figure
230 1Cii-iii). During the night, *ninaE* hypomorphs both show increased sleep (Figure 1Gii-iii), but only
231 $ninaE^{8}$ mutant shows significant increase of the average sleep bout length compared to control flies
232 (Figure 1Eii-iii). However, mutants severely reduce their waking activity (Figure 1Fii-iii with effect size
233 *r* between 0.67 and 0.82, Table 1). Therefore, the increase in night sleep observed here cannot be
234 certain, and may simply reflect the loss of locomotor activity. To confirm *ninaE* mutants harbour
235 phototransduction defects, we measured light-evoked receptor potentials through
236 Electroretinograms (ERG, Figure 1H). Consistent with previous reports, $ninaE^{17}$ and $ninaE^{8}$ exhibit
237 reduced light induction of receptor potential, on and off transients (Figure 1I-J), whereas $ninaE^{5}$
238 retains certain function (see normal receptor potential and off-transient in $ninaE^{5}$, Figure 1J). Taken
239 together, our data indicates that the reduction, or loss of, *Drosophila* Rhodopsin 1 (ninaE) protein
240 results in the reduction of day sleep bout length, and that phototransduction is required to drive day
241 sleep pressure. However, recent studies show support for a non-visual function of ninaE (Zanini et
242 al., 2018). To verify if ninaE protein alone or the phototransduction in general regulates day sleep
243 bout length, we further examined the sleep-regulatory roles for three other key proteins for
244 phototransduction.

245 **3.1.2 *norpA***

246 Metarhodopsin triggers Gqα activation and enzymatic reaction of Phospholipase C (PLC) that
247 produces secondary messengers. The norpA protein is the main PLC in the visual system, and the
248 classic mutant $norpA^{p24}$ exhibits severe visual defects (Gao et al., 2008). The sleep profile of $norpA^{p24}$
249 shows decline of day sleep from the midday and a secondary dip at midnight compared to the
250 control (Figure 2A). There is also a phase advance of night sleep in $norpA^{p24}$ mutants as compared to
251 the controls. Such an advance was also noticeable in locomotor profile of another *norpA* allele in a
252 previous study (Ogueta et al., 2018). The changes in sleep profile coincides with the reduction in day
253 sleep (Figure 2D) and shorter bout length in both day and night (Figure 2B, 2E). Similar to $ninaE^{5}$ and
254 $ninaE^{8}$, the $norpA^{p24}$ mutant has low waking activity (Figure 2C, 2F, effect size 0.39/0.75, Table 2). We
255 also confirmed abnormal ERG profiles in $norpA^{p24}$ (Figure 2H).

256 **3.1.3 *trp and trpl***

257 Activation of the two ion channels, trp and trpl in the last step of phototransduction causes the
258 crucial cation influx resulting in depolarisation of the photoreceptor. We therefore examined the
259 sleep profile of four mutant lines, two P-element insertion mediated mutants: trp^{MB}, $trpl^{MB}$ (Chen et

260 al., 2015) and two classic null mutants: *trp*^{1}, and *trpl*^{302} (Cosens and Manning, 1969; Leung et al.,
261 2000; Niemeyer et al., 1996). Except for *trp*^{1}, these lines have white eyes, like the *ninaE* and *norpA*
262 mutants as described above.

263 The *trp*^{1} mutant was first isolated from a red eye wild type strain, but the precise background was
264 not known (Cosens and Manning, 1969), so we compared its sleep profile to two widely used wild
265 type strains in sleep and vision research: *Canton S* and *Oregon R.* Significant reductions in day and
266 night sleep and average sleep bout length are shown in *trp*^{1} mutants as compared to either wild type
267 flies (Figure 3Ai, 3Bi and 3Ei, effect size: 0.29-0.54, Table 3). The waking activity of *trp*^{1} is comparable
268 to *Canton S* but higher than *Oregon R*, and has limited impact to sleep loss (Figure 3Ci and 3Fi). The
269 sleep profile of *trp*^{MB} is distinct from *trp*^{1}*, norpA* or *ninaE* mutants, with day sleep level similar to
270 controls but a significant night sleep loss (Figure 3Aii, 3Gii). There is no significant change in sleep
271 bout length detected in *trp*^{MB} flies (Figure 3Aii and 3Eii). Although, *trp*^{MB} flies sleep a similar amount
272 during the day, compared to the control, their waking activity is significantly higher (Figure 3Cii).

273 This relative normal sleep-wake profile may relate to the less severe reduction in receptor potential
274 and light on/off transients in the ERG of *trp*^{MB} as compared to those of the *trp*^{1} null mutant (Figure
275 3Hi and 3Hii, effect size r = 0.11-0.83 vs 0.78-0.85, Table 3).

276 On the other hand, both *trpl*^{302} and *trpl*^{MB} mutants exhibit day sleep profiles similar to *ninaE* or
277 *norpA* mutants (Figure 3Aiii and 3Aiv), with significant reduced day sleep and average bout length
278 (Figure 3Biii, 3Biv, 3Diii and 3Div). Night sleep profiles and waking activities for the two *trpl* mutants
279 diverged: *trpl*^{302} sleep more whereas *trpl*^{MB} sleep less (Figure 3Giii, 3Giv). No significant changes in
280 night sleep bout length were observed in both mutants as compared to the controls (Figure 3Eiii and
281 3Eiv). *trpl*^{302} mutants generally have low waking activity, whereas *trpl*^{MB} maintains relative normal
282 waking activity as compared to the controls (Figure 3Ciii, 3Civ, 3Fiii and 3Fiv). *trpl*^{302} mutant is in *cn*
283 and *bw* mutation background, which may affect the sleep. We therefore tested *w; trpl*$^{302/MB}$ flies to
284 reduce the impact of *cn bw* mutation. Similar to *trpl*^{302} and *trpl*^{MB}, the *w; trpl*$^{302/MB}$ transheterozygote
285 mutant showed shorter day sleep and sleep bout length (Figure 3Biv). As seen in previous reports
286 (Chen et al., 2015; Leung et al., 2000) both mutants have limited impact on ERG profiles, *trpl*^{302} flies
287 show a large reduction of on/off transient (effect size 0.73/0.81, Table 4), whereas *trpl*^{MB} mutants
288 maintain normal ERGs, possibly due to synaptic compensatory mechanism (Figure 3Hiii).
289 Nevertheless, our data indicate the reduced trpl function and expression level cause sleep loss in the
290 day.

291 **3.2 Divergent sleep function in histaminergic pathway**

292 *Drosophila* photoreceptors are divided into Rh1-expressing (R1-R6) and non-Rh1-exppressing
293 neurons (R7/R8). The R1-R6 photoreceptors form synapses onto lamina monopolar neurons (LMC),
294 while the R7/R8 send their output to the medulla in the optic lobes. Histamine is the primary
295 neurotransmitter for Rh1-expessing photoreceptors. Therefore, we tested if mutants with defects in
296 histamine synthesis or postsynaptic reception show altered sleep. Histidine decarboxylase (*hdc*) is
297 the main histamine synthesis enzyme (Pantazis et al., 2008). We investigated the sleep profiles for
298 the two *hdc* mutants (hdc^{JK910} and hdc^{MB}) that were showed to have reduced histamine levels in the
299 photoreceptors (Burg et al., 1993; Stenesen et al., 2015).

300 As expected, both mutants selectively reduce on/off transients' levels, the components in ERG that
301 reflect synaptic transmission between photoreceptors and LMCs (Figure 4Hi). Surprisingly, significant
302 sleep defects were detected in the night instead of during the day. During the day, increased day
303 sleep and bout length are found in hdc^{JK910} mutants as compared to the controls, whereas hdc^{MB} flies
304 show comparable day sleep and bout length to the controls (Figure 4Bi-ii and 4Di-ii). The waking
305 activities in the day are similar between *hdc* mutants and controls (Figure 4Ci-ii). During the night,
306 both *hdc* mutants sleep less with shorter sleep bouts (Figure 4Ei-ii and 4Gi-ii). Only hdc^{JK910} mutants
307 have reduced waking activities compared to the controls at night (Figure 4Fi-ii). Two histamine
308 receptors, ort and HisCl1, are detected in *Drosophila* CNS, and they are both histamine-gated
309 chloride channels (Pantazis et al., 2008). Transcriptomic data in *Drosophila* visual system suggests
310 that ort is expressed selectively in neurons postsynaptic to photoreceptors, whereas HisCl1 is
311 expressed almost exclusively in photoreceptors as auto-receptors (Davis et al., 2020). We therefore
312 verified the sleep profile in ort mutants to investigate the role of histamine signal downstream to
313 photoreceptors.

314 First, we monitored the sleep profile of the classic ort^{1} mutant against *Canton S*, as they share the
315 same genetic backgrounds (Inoue et al., 1989). A small but significant day time sleep loss is detected
316 in ort^{1} flies (Figure 4Dii, effective size 0.27, Table 5). ort^{1} mutants sleep for longer bouts during the
317 day but have higher day waking activity as compared to *Canton S* (Figure 4Biii and 4Ciii). In contrast
318 to *hdc* mutants, no night sleep phenotype is detected in ort^{1} flies (Figure 4Eiii-4Giii). We also tested
319 a recent CRISPR-mediated null mutation ort^{attp} in the white eye background (Deng et al., 2019). The
320 sleep profile of ort^{attp} is distinct from ort^{1}, showing more sleep as compared to control (Figure 4Div
321 and 4Giv). However, the waking activities of ort^{attp} are severely reduced (Figure 4Civ and 4Fiv,
322 effective size 0.55/0.60, Table 5) therefore compounding the observed sleep phenotype. Intriguingly,
323 shorter day sleep bout length and a small reduction of night sleep are detected in $ort^{attp/+}$
324 heterozygotes (Figure 4Biv and 4Giv), accompanying improved waking activity (Figure 4Civ and 4Fiv,
325 wake activity ort^{attp} vs $ort^{attp/+}$, Table 5).

326 Both *ort* mutants exhibit ERG profiles with loss of on/off transients, while ort^{1} mutants also show
327 loss of the receptor potential (Figure 4Hii).

328 **3.3. Hyperpolarisation of Rh1-expressing photoreceptor causes short day sleep bout**

329 Our findings so far indicate that seven of the eight phototransduction mutants exhibit day time sleep
330 defects. However, day sleep loss in $trpl^{MB}$ mutants do not correlate with ERG phenotypes. Moreover,
331 $norpA^{p24}$, trp^{MB}, $trpl^{MB}$, $ort^{attp/+}$, and *hdc* mutants show sleep loss in the night. Thus, we further tested
332 if direct inhibition of photoreceptor excitability causes sleep defects in the day or night. At first, we
333 applied the Gal4-UAS system to block synaptic transmission from all photoreceptors by expressing
334 UAS-TeTxLC (tetanus toxin light chain) with gmr-Gal4 (as seen in Damulewicz et al., 2020). However,
335 we were not able to obtain viable adult flies even after maintaining the culture in 22°C to reduce the
336 known overexpression-related toxicity (Rezával et al., 2007; Sweeney et al., 1995). We next used
337 UAS-Kir2.1 (Baines et al., 2001; Nitabach et al., 2002) crossed with gmr-Gal4 or Rh1-Gal4 to
338 hyperpolarise all or just Rh1-expressing photoreceptors. Consistent with the finding for *ninaE* and
339 *norpA* mutants, *gmr-Gal4>uas-Kir2.1* and *Rh1-Gal4>uas-Kir2.1* flies showed reduced day sleep as
340 compared to *Gal4>+* or *+>uas-Kir2.1* controls (Figure 5Ai-ii, 5Di-ii), but only *Rh1-Gal4>uas-Kir2.1* flies
341 exhibit reduced sleep bout length in the day (Figure 5Bi-ii). Night sleep parameters in both *gmr-*
342 *Gal4>uas-Kir2.1* and *Rh1-Gal4>uas-Kir2.1* flies ranges between that of the controls (Figure 5E-G).
343 This is because the unexpected low sleep exhibited in *+>uas-Kir2.1* controls (*gmr-Gal4>+* vs *+>uas-*
344 *Kir2.1* and *Rh1-Gal4>+* vs *+>uas-Kir2.1*; effect size 0.62/0.74, Table 6). Therefore, we cannot
345 determine the actual effect of hyperpolarising photoreceptor on night sleep. Kir2.1 expressing flies
346 had no changes in waking activity (Figure 5C and 5F). Taken together, our data indicates that
347 phototransduction and the neuronal excitability in the *Drosophila* Rh1-expressing outer
348 photoreceptors promote day sleep.

349 **4. Discussion**

350 **4.1 Primary finding**

351 In this study, we surveyed eight mutants with defects in phototransduction, four mutants with
352 disrupted histaminergic transmission, the main neurotransmitter used by *Drosophila*
353 photoreceptors, and two mutants with hyperpolarised photoreceptors. We observed short or
354 fragmented day sleep in the majority of the phototransduction mutants as well as in mutants with
355 hyperpolarised R1-R6 photoreceptors. This data indicates that R1-R6 photoreceptor neural activities
356 are required for sleep homeostatic drive during the daytime. Importantly, the mutants tested in this
357 study maintain their ability to sense light, as the non-visual pathways such as *cry*, *Rh7* and *qsm*
358 remain intact. Nevertheless, we show robustly that reduced visual phototransduction, or
359 downstream neurotransmission, cause sleep loss and/or sleep fragmentation, indicating a clear role
360 for *Drosophila* photoreceptors R1-R6 in promoting day sleep.

361 **4.2 Contradiction to previous studies**

362 Our data contradicts previous findings. Specifically, it was previously shown that blocking neural
363 transmission for R1-R6 photoreceptors via TeTxLC caused day sleep increase (Damulewicz et al.,
364 2020). As mentioned above, we cannot produce viable adult flies that express TeTxLC in
365 photoreceptors and instead we applied Kir2 channel to hyperpolarise retinal photoreceptors. There
366 may be two factors that explain the data discrepancy between the two studies: Firstly, our Gal4 and
367 UAS lines are outcrossed to the w^{iso31} background, as is standard in the sleep research field, while
368 such outcrossing was not described in Damulewicz et al., (2020). Secondly, vision is important for
369 navigation, and the compromised vision in phototransduction mutants and flies with weakened
370 photoreceptor outputs (i.e., *gmr-Gal4>UAS-kir2* or *gmr-Gal4>UAS-TeTxLC*) can result in reduced
371 waking locomotor activities, which is a major confounding factor in assessing sleep increase. In fact,
372 eight visual mutants in our survey (*ninaE*^{5}, *ninaE*^{8}, *norpA*^{p24}, *trp*^{1}, *trpl*^{302}, *hdc*^{JK910}, *ort*^{attp} and *gmr-*
373 *Gal4>uas-kir2*) showed significant reduced waking activity in either day and/or night.
374 Consequentially, the reduced waking activity prevents us from concluding whether the increased
375 night sleep seen in *ninaE*^{8}, *trpl*^{302} and *ort*^{attp} are genuine increases or a reflection of loss of
376 locomotion during wakefulness. Therefore, these night sleep increase phenotypes should be
377 interpreted with caution. The waking activity was not analysed by Damulewicz et al., (2020)
378 preventing direct comparison here.

379 More surprisingly, the sleep loss identified in *hdc* and *ort* mutants is also opposite to previous
380 findings indicating that histamine is a pro-waking factor (Oh et al., 2013). One major difference

381 between the current study and Oh et al., (2013) is that we only monitored male sleep to reach a
382 larger range of sleep dynamics. Importantly, female flies have very low levels of day sleep (see 2.2 in
383 Materials and method). As mentioned, further investigations of female visual mutants are necessary
384 before it can be determined if the role of visual input to sleep diverges between male and female
385 flies. Nevertheless, as shown in our data, two of the four mutants with histaminergic disruption
386 show lower waking activity, consistent with the proposed arousal promoting role of histamine (Oh et
387 al., 2013). However, we note that the mentioned previous study (Oh et al., 2013) also did not assess
388 the impact of waking activity on sleep. Waking activity is a classic, but often ignored, analytical tool
389 in *Drosophila* sleep research that allows differentiation between sleep and locomotor defects: as we
390 demonstrate in this study, when the flies exhibit reduced locomotor activity, any increase in sleep
391 cannot be certain. We therefore recommend that this parameter always be included in any
392 locomotion-based sleep assay.

393 **4.3 Experimental limitations**

394 The unexpected abnormal low night sleep in *+>uas-Kir2* control flies which is likely due to leaky
395 expression of Kir2 in other parts of the fly brain, since the *gmr-Gal*4 and *UAS-Kir2* lines are
396 isogenised to w^{iso31} background. Nevertheless, this limits our investigation to day sleep. Future
397 investigation using gmr-LexA and LexAoP-Kir2 may be required to verify this finding.

398 The visual mutants tested in this study come with various genetic backgrounds including unknown
399 *white* backgrounds and two wild type backgrounds (see Materials and methods). We therefore only
400 interpreted the consistent phenotypes presented across the alleles. As such, there are allelic specific
401 night sleep loss phenotypes in in trp^{MB}, $trpl^{MB}$, $ort^{attp/+}$ and $norpA^{p24}$ that is likely due to genetic
402 background and should be interpreted with caution.

403 As part of control experiments, we examined the ERG profiles for all the tested gene-specific
404 mutants. However, the severity of sleep loss phenotype does not directly correlate with ERG defects
405 except for *trp* mutants: the weakest $ninaE^{5}$ mutant (only on transient reduction was detected)
406 showed just as much day sleep fragmentation as $ninaE^{8}$, $ninaE^{17}$ or $norpA^{p24}$. Moreover, both $trpl^{302}$
407 and $trpl^{MB}$ exhibit sleep loss, despite minor or no impact of *trpl* mutants on ERG profile. The
408 divergent sleep phenotypes may be partly due to the various genetic backgrounds that we did not
409 control for (see Materials and methods). Moreover, it is important to note that ERG only reflects
410 outer-retina physiology, not downstream optic-lobe integration. Therefore, the inconsistent sleep
411 phenotypes can also be derived from the expression profile of phototransduction genes in the
412 central brain beyond the photoreceptors. Future investigation utilising isogenised somatic CRISPR

413 knockout constructs will be required to verify potential impacts of genetic background on the
414 identified sleep-controlling role of phototransduction.

415 **4.4 Hypothetical mechanisms underlying day and night sleep**

416 Vision and light-affected sleep are mediated both via circadian clock neurons (eg., LNvs and DN1) as
417 well as by ring neurons in the EB (Lamaze et al., 2018; Raccuglia et al., 2019; Shang et al., 2008; Singh
418 et al., 2023). Various neural pathways connect photoreceptors to the wake promoting LNv: R1-R6
419 photoreceptors propagate the light signal via histamine-mediated inhibition of the downstream LMC
420 neurons that activates LNvs (Muraro and Ceriani, 2015); on the other hand, R8 has recently been
421 shown to communicate with LNv via the so-called AMA neurons and cholinergic signalling.
422 Moreover, sleep promoting signals from photoreceptors may also reach the EB via the Anterior
423 Visual Pathway through MeTu and TuBu neurons, upon which circadian clock neuron DN1 also
424 converge (Guo et al., 2018; Lamaze et al., 2018). It is currently unclear which of the above pathways
425 are required for the photoreceptor mediated day sleep promotion (Figure 6).

426 Although day sleep loss in *ort*^{1}, *ninaE* mutants and *Rh1-Gal4>uas-Kir2* indicates that day sleep
427 depends on Rh1-positive R1-R6-mediated histaminergic inhibition on LMC neurons, we did not
428 observe day sleep loss among *hdc* mutants. Nevertheless, hdc expression is wider than the visual
429 system (Oh et al., 2013). Moreover, loss of hdc activity does not completely remove histamine
430 production (eg., Stenesen et al., 2015) and it is not equal to selective blockage of ort-mediated
431 histamine postsynaptic transmission. However, our findings are consistent with those that previously
432 found a visually impaired *omb* mutant showed major sleep loss in both day and night (Kirszenblat et
433 al., 2019) as well as the finding that activation of L2 LMC neurons cause day sleep lost (Muraro and
434 Ceriani, 2015). Moreover, our data fit well with previous studies predicting that medium intensity
435 light convey a day sleep signal via retinal photoreceptors, whereas high intensity light enhances
436 midday sleep via HB-eyelet (Schlichting et al., 2019). Future orthogonal circuit-manipulation
437 experimentation is required to verify if the R1-R6 photoreceptors-mediated day sleep depends on L2
438 LMC and/or LNvs (Figure 6).

439 Six tested mutants also exhibit night sleep loss (two *hdc* mutants, *norpA*^{p24}, *trp*^{1}, *trp*^{MB} and *trpl*^{MB}).
440 Genetic background variation may contribute to this night sleep loss. However, both *hdc* mutants
441 exhibit clear night specific sleep loss and *hdc*^{MB} mutants are in an isogenic *w*^{iso31} background.
442 Therefore, this night sleep loss is likely genuine. Day time visual experience influence night sleep in
443 flies despite there is no or limited vision in the darkness of the night. There are three possible
444 explanations for night sleep loss in *hdc* mutants: **1.** Night sleep loss can simply be caused by non-
445 retinal photoreceptor histaminergic neurons and is mediated by HisCl1 reception instead of ort (Oh

446 et al., 2013). **2.** Reduction of photoreceptor-mediated histamine results in uneven inhibition among
447 LMCs to reduce the visual contrast of bright/dark edges and differentially reduce activation of HS/VS
448 neurons which are known promote night sleep (Kirszenblat et al., 2019). **3.** The *hdc*-mediated night
449 sleep is related to the reduced histaminergic inhibition in non-LMC neurons mediated by HisCl1 and
450 is independent of ort. Previous data indicates that HisCl1 is an auto-receptor for all retinal
451 photoreceptors, and also expresses in glia cells in the optic lobe as well as clock neurons lLNvs (Davis
452 et al., 2020; Oh et al., 2013; Pantazis et al., 2008). Among retinal photoreceptors, R8 has been
453 proposed to receive histaminergic inhibition via HisCl1 to regulate its acetylcholine co-release
454 (Alejevski et al., 2019; Xiao et al., 2023). Loss of this inhibition can result in an ectopic increase in
455 acetylcholine, which can in turn activate the newly identified AMA (Xiao et al., 2023) and its
456 downstream wake promoting lLNvs (Shang et al., 2008). Alternatively, R8 can reach sleep/wake
457 promoting EB-R via anterior visual pathways (Figure 6).

458 The potential demarcation between achromatic (R1-R6) and chromatic (R8, both blue and green
459 sensitive) photoreceptors in their control over day and night sleep (Figure 6) bears some
460 resemblance with the previous finding in mammalian system, where the sleep- vs wake-promoting
461 effects of green and blue light required proper neural output from rod/cone and the melanopsin
462 expressing photosensitive retinal ganglion cells (Pilorz et al., 2016). Nevertheless, the revelation of
463 the potential neural network demarcation of day and night sleep in *Drosophila* require further
464 investigation to expand our understandings in circuital logic of light-mediated sleep regulation.

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Author Contributions

K-F.C – Conceptualization, Methodology, Investigation, Writing - Original Draft, Writing – Review & Editing, Visualization, Supervision, Project administration, Funding acquisition; Y.-C. H. – Investigation, Visualization, Writing – Review & Editing. B.W. – Methodology, Investigation, Writing – Review & Editing. M.A., X.L., N. S., S. H., T. O., J. A, and C. S.-G., – Investigation, Writing – Review & Editing.

Declaration Of Interests

The authors declare no competing interests.

481 **Figure captions**

482 **Figure 1. sleep profile, parameters and ERG quantification for *ninaE* mutants. A.** Sleep traces of
483 average sleep minutes per 30 minutes over 12 hours light-dark cycle. ZT0: light on, ZT12: light off,
484 ZT24: end of night. Sleep traces of *iso* control and *ninaE* mutants are colour coded in grey and red
485 shades (i-iii). **B.** Average day sleep bout length (minutes), **C.** Average day wake activity (beam breaks
486 per waking minutes), **D.** Average day sleep (minutes), E. Average night sleep bout length (minutes),
487 **F.** Average night wake activity (beam breaks per waking minutes), and **G.** Average night sleep
488 (minutes) for $ninaE^{17}$ (i), $ninaE^{8}$ (ii) and $ninaE^{5}$ (iii) compared to iso controls. Average values for each
489 individual are plotted as filled circles except for those outside scales. **H.** An example ERG profile from
490 iso control with 1000 ms green light flash that evokes voltage changes of on-transient, receptor
491 potential and off-transient. **I.** Mean and standard deviation for voltage change (mV) in on transient,
492 receptor potential and off-transient of ERG upon four light intensity (0.004, 0.02, 0.1 and 0.5 w/m^2)
493 flash for $ninaE^{17}$ and controls. **J.** Mean and standard deviation for voltage change (mV) in on
494 transient, receptor potential and off-transient of ERG upon four light intensities (0.004, 0.02, 0.1 and
495 0.5 w/m^2) 1000ms flash for $ninaE^{5}$, $ninaE^{8}$ and controls. Plot scales are in either liner (A-G) and
496 logarithmic (I-J). Asterisk indicates statistical significance ($p<0.05$) between mutants and iso controls
497 by pairwise Wilcoxon rank sum exact test without (B-H) and with multiple comparison, Benjamini-
498 Hochberg correction (I, followed by Kruskal-Wallis rank sum test). See **Table 1** for sample size (n=26-
499 60 for sleep; n=3-6 for ERG, with 4 replicates for each light intensity), p-values and effect size for
500 each comparison.

501 **Figure 2. sleep profile, parameters and ERG quantification for *norpA* mutants. A.** Sleep traces of
502 average sleep minutes per 30 minutes over 12 hours light-dark cycle. ZT0: light on, ZT12: light off,
503 ZT24: end of night. Sleep traces for *iso* control and $norpA^{p24}$ mutants are colour coded in grey and
504 cyan. **B.** Average day sleep bout length (minutes), **C.** Average day wake activity (beam breaks per
505 waking minutes), **E.** Average night sleep bout length (minutes), **F.** Average night wake activity (beam
506 breaks per waking minutes), **G.** Average night sleep (minutes) for $norpA^{p24}$ compared to iso controls.
507 Average values for each individual are plotted as filled circles except for those outside scales (B-G).
508 **H.** mean and standard deviation for voltage change (mV) in on transient, receptor potential and off-
509 transient of ERG upon four light intensity (0.004, 0.02, 0.1 and 0.5 w/m^2) flash for $norpA^{p24}$ and *iso*
510 controls. Plot scales are in either liner (A-G) and logarithmic (H). Asterisk indicates statistical
511 significance ($p<0.05$) between mutants and iso controls by pairwise Wilcoxon rank sum exact test.
512 See **Table 2** for sample size (n=21-23 for sleep; n=4 for ERG, with 4 replicates for each light
513 intensity), p-values and effect size for each comparison.

514 **Figure 3. sleep profile, parameters and ERG quantification for *trp* and *trpl* mutants. A.** Sleep traces
515 of average sleep minutes per 30 minutes over 12 hours light-dark cycle. ZT0: light on, ZT12: light off,
516 ZT24: end of night. Sleep traces for *Canton S* (i), *Oregon R* (i) and *iso* controls (ii-iv) are coloured
517 coded in the shades of greys; *trp* (i-ii) and *trpl* (iii-iv) mutants are colour coded in shades of blue and
518 purples. **B.** Average day sleep bout length (minutes), **C.** Average day wake activity (beam breaks per
519 waking minutes), **D.** Average day sleep (minutes), **E.** Average night sleep bout length (minutes), **F.**
520 Average night wake activity (beam breaks per waking minutes), **G.** Average night sleep (minutes) for
521 trp^{1} (i), trp^{MB} (ii), $trpl^{302}$ (iii) and $trpl^{302/MB}$ or $trpl^{MB}$ (iv) compared to controls. Average values for each
522 individual are plotted as filled circles except for those outside scales. **H.** Mean and standard
523 deviation for voltage change (mV) in on transient, receptor potential and off-transient of ERG upon
524 four light intensity (0.004, 0.02, 0.1 and 0.5 w/m^2) flash for trp^{1} (i), trp^{MB} (ii), $trpl^{302}$ (iii) and $trpl^{MB}$ (iii)
525 compared to controls. Plot scales are in either liner (A-G) and logarithmic (H). Asterisk indicates
526 statistical significance ($p<0.05$) between mutants and controls by pairwise Wilcoxon rank sum exact
527 test without (Aii-Gii, Aiii-Giii, Hi and Hii) and with multiple comparison, Benjamini-Hochberg
528 correction (Ai-Gi, Aiv-Giv, and Hiii followed by Kruskal-Wallis rank sum test). See **Table 3 and Table 4**
529 for sample size (n=16-53 for sleep; n=3 for ERG, with 4 replicates for each light intensity), p-values
530 and effect size for each comparison.

531 **Figure 4. sleep profile, parameters and ERG quantification for *hdc* and *ort* mutants. A.** Sleep traces
532 of average sleep minutes per 30 minutes over 12 hours light-dark cycle. ZT0: light on, ZT12: light off,
533 ZT24: end of night. Sleep traces for *iso* (i-ii, iv) and *Canton S* (iii) controls are coloured coded in the
534 shades of greys; *hdc* (i-ii) and *ort* (iii-iv) mutants are colour coded in shades of indigos and greens. **B.**
535 Average day sleep bout length (minutes), **C.** Average day wake activity (beam breaks per waking
536 minutes), **D.** Average day sleep (minutes), **E.** Average night sleep bout length (minutes), **F.** Average
537 night wake activity (beam breaks per waking minutes), **G.** Average night sleep (minutes) for *hdc*
538 mutants (i-ii), ort^{1} and $ort^{attp/+}$ or ort^{attp} (iii-iv) compared to controls. Average values for each
539 individual are plotted as filled circles except for those outside scales (B-G). **H.** Mean and standard
540 deviation for voltage change (mV) in on transient, receptor potential and off-transient of ERG upon
541 four light intensity (0.004, 0.02, 0.1 and 0.5 w/m^2) flash for *hdc* (i) and *ort* (ii) mutants compared to
542 controls. Plot scales are in either liner (A-G) and logarithmic (H). Asterisk indicates statistical
543 significance ($p<0.05$) between mutants and controls by pairwise Wilcoxon rank sum exact test
544 without (Aii-Gii) and with multiple comparison, Benjamini-Hochberg correction (Ai-Hi, Aiii-Giii, and
545 Hii followed by Kruskal-Wallis rank sum test). See **Table 5** for sample size (n=29-60 for sleep; n=3-5
546 for ERG, with 4 replicates for each light intensity), p-values and effect size for each comparison.

547 **Figure 5. sleep profile and parameters for flies with hyperpolarised photoreceptors. A.** Sleep traces
548 of average sleep minutes per 30 minutes over 12 hours light-dark cycle. ZT0: light on, ZT12: light off,
549 ZT24: end of night. Sleep traces for *gmr-Gal4>+* (i), *Rh1-Gal4>+* (ii) and *+>UAS-Kir2* (i-ii) control flies
550 are coloured coded in the shades of greys; experimental mutants *gmr-Gal4> UAS-kir2* (i) and *Rh1-*
551 *Gal4>UAS-kir2* (ii) are colour coded in light burgundy. **B.** Average day sleep bout length (minutes), **C.**
552 Average day wake activity (beam breaks per waking minutes), **D.** Average day sleep (minutes), **E.**
553 Average night sleep bout length (minutes), **F.** Average night wake activity (beam breaks per waking
554 minutes), **G.** Average night sleep (minutes) for *gmr-Gal4> UAS-kir2* (i) and *Rh1-Gal4>UAS-kir2* (ii)
555 compared to controls. Average values for each individual are plotted as filled circles except for those
556 outside scales (B-G). Asterisk indicates statistical significance ($p<0.05$) between mutants and controls
557 by pairwise Wilcoxon rank sum exact test with multiple comparison, Benjamini-Hochberg correction
558 (followed by Kruskal-Wallis rank sum test). See **Table 6** for sample size (n=28-32), p-values and effect
559 size for each comparison.

560 **Figure 6. Speculative mechanism underlying visually driven day and night sleep.** R1-R6
561 photoreceptors (blue) promote day sleep by inhibiting a subset of wake promoting lamina
562 monopolar cells (LMC) via histamine release (his, blue text) and ort reception (green text); Night
563 sleep is controlled by interaction between R1-R6, R7 and R8 (blue/orange) photoreceptors:
564 Hypothetical histaminergic inhibition (his, blue text) from R1-R6 to R8 is mediated by HisCl1
565 (magenta text) (Alejevski et al., 2019). Separately R7 and R8 also inhibit each other via histaminergic
566 signalling (Schnaitmann et al., 2018), these inhibitions maintain a slower suppression of
567 acetylcholine release (ace, orange text) from R8 and its activation of the AMA neurons (orange) and
568 downstream waking promoting LNvs (red circle). Alternatively, R8 regulate night sleep by reaching
569 sleep-regulating EB-Ring neurons (EB-R) via MeTu and TuBu neurons with unknown
570 neurotransmitters (grey rectangular and arrows).

571

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Table 1. Statistics of a. sleep and b. ERG parameters for *ninaE* mutants

a. sleep parameter	genotype comparison	p-value	effect size *r*	n1	n2	significant *r* magnitude
day sleep	*iso* vs *ninaE*^{17}	3.50E-01	0.09	60	52	
night sleep	*iso* vs *ninaE*^{17}	1.00E-07	0.51	60	51	large
day sleep bout length	*iso* vs *ninaE*^{17}	9.60E-06	0.42	60	52	moderate
night sleep bout length	*iso* vs *ninaE*^{17}	7.90E-01	0.03	44	35	
day wake activity	*iso* vs *ninaE*^{17}	5.70E-01	0.05	60	52	
night wake activity	*iso* vs *ninaE*^{17}	1.30E-01	0.14	60	51	
day sleep	*iso* vs *ninaE*^{5}	1.40E-03	0.40	32	31	moderate
night sleep	*iso* vs *ninaE*^{5}	3.20E-02	0.27	32	31	small
day sleep bout length	*iso* vs *ninaE*^{5}	5.30E-09	0.68	32	31	large
night sleep bout length	*iso* vs *ninaE*^{5}	7.10E-02	0.23	32	31	
day wake activity	*iso* vs *ninaE*^{5}	3.90E-13	0.79	32	31	large
night wake activity	*iso* vs *ninaE*^{5}	1.90E-10	0.72	32	31	large
day sleep	*iso* vs *ninaE*^{8}	9.30E-05	0.51	32	26	large
night sleep	*iso* vs *ninaE*^{8}	1.10E-08	0.75	32	26	large
day sleep bout length	*iso* vs *ninaE*^{8}	6.90E-09	0.76	32	26	large
night sleep bout length	*iso* vs *ninaE*^{8}	9.60E-02	0.22	32	26	
day wake activity	*iso* vs *ninaE*^{8}	1.40E-13	0.82	32	26	large
night wake activity	*iso* vs *ninaE*^{8}	2.90E-07	0.67	32	26	large
b.ERG parameter	**genotype comparison**	**p-value**	**effect size *r***	**n1**	**n2**	**significant *r* magnitude**
receptor potential	*iso* vs *ninaE*^{17}	2.50E-06	0.71	6*4	3*4	large
on transient	*iso* vs *ninaE*^{17}	1.90E-08	0.78	6*4	3*4	large
off transient	*iso* vs *ninaE*^{17}	1.60E-09	0.81	6*4	3*4	large
receptor potential	*iso* vs *ninaE*^{5}	4.40E-01	0.17	3*4	3*4	
receptor potential	*iso* vs *ninaE*^{8}	4.40E-06	0.83	3*4	3*4	large
receptor potential	*ninaE*^{5} vs *ninaE*^{8}	4.40E-06	0.84	3*4	3*4	large
on transient	*iso* vs *ninaE*^{5}	1.70E-03	0.64	3*4	3*4	large
on transient	*iso* vs *ninaE*^{8}	4.40E-06	0.84	3*4	3*4	large
on transient	*ninaE*^{5} vs *ninaE*^{8}	5.97E-02	0.39	3*4	3*4	
off transient	*iso* vs *ninaE*^{5}	1.70E-02	0.48	3*4	3*4	moderate
off transient	*iso* vs *ninaE*^{8}	1.10E-06	0.85	3*4	3*4	large
off transient	*ninaE*^{5} vs *ninaE*^{8}	1.10E-06	0.85	3*4	3*4	large

n1 = sample size for first genotype in genotype comparison; **n2** = sample size for first genotype in genotype comparison; * sample size in ERG statistic is actual sample size multiple with four reflecting the values from the four light intensities

841 **Table 2. Statistics of a. sleep and b. ERG parameters for *norpA* mutants**

a. sleep parameter	genotype comparison	p-value	effect size *r*	n1	n2	significant *r* magnitude
day sleep bout length	*iso* vs $norpA^{p24}$	9.40E-03	0.39	23	21	moderate
night sleep bout length	*iso* vs $norpA^{p24}$	2.50E-06	0.66	23	21	large
day sleep	*iso* vs $norpA^{p24}$	3.20E-02	0.32	23	21	moderate
night sleep	*iso* vs $norpA^{p24}$	1.20E-01	0.23	23	21	
day wake activity	*iso* vs $norpA^{p24}$	9.40E-03	0.39	23	21	moderate
night wake activity	*iso* vs $norpA^{p24}$	1.70E-07	0.72	23	21	large
b.ERG parameter	**genotype comparison**	**p-value**	**effect size *r***	**n1**	**n2**	**significant *r* magnitude**
receptor potential	*iso* vs $norpA^{p24}$	1.50E-06	0.85	4*4	4*4	large
on transient	*iso* vs $norpA^{p24}$	3.30E-09	0.85	4*4	4*4	large
off transient	*iso* vs $norpA^{p24}$	1.50E-06	0.85	4*4	4*4	large

n1 = sample size for first genotype in genotype comparison; **n2** = sample size for first genotype in genotype comparison; * sample size in ERG statistic is actual sample size multiple with four reflecting the values from the four light intensities

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Table 3. Statistics of a. sleep and b. ERG parameters for *trp* mutants

a. sleep parameter	genotype comparison	p-value	effect size *r*	n1	n2	significant *r* magnitude
day sleep	*Canton S* vs *Oregon R*	5.33E-01	0.07	38	53	
day sleep	*Canton S* vs trp^{1}	1.50E-04	0.47	38	30	moderate
day sleep	*Oregon R* vs trp^{1}	2.90E-06	0.54	53	30	large
night sleep	*Canton S* vs *Oregon R*	1.20E-04	0.41	38	53	moderate
night sleep	*Canton S* vs trp^{1}	1.63E-02	0.29	38	30	small
night sleep	*Oregon R* vs trp^{1}	2.30E-07	0.59	53	30	large
day sleep bout length	*Canton S* vs *Oregon R*	8.66E-01	0.02	38	53	
day sleep bout length	*Canton S* vs trp^{1}	1.40E-02	0.32	38	30	moderate
day sleep bout length	*Oregon R* vs trp^{1}	1.20E-02	0.32	53	30	moderate
night sleep bout length	*Canton S* vs *Oregon R*	6.61E-01	0.05	38	53	
night sleep bout length	*Canton S* vs trp^{1}	5.20E-03	0.35	38	30	moderate
night sleep bout length	*Oregon R* vs trp^{1}	1.20E-03	0.39	53	30	moderate
day wake activity	*Canton S* vs *Oregon R*	8.20E-08	0.58	38	53	large
day wake activity	*Canton S* vs trp^{1}	7.70E-01	0.04	38	30	
day wake activity	*Oregon R* vs trp^{1}	2.90E-07	0.57	53	30	large
night wake activity	*Canton S* vs *Oregon R*	1.20E-07	0.58	38	53	large
night wake activity	*Canton S* vs trp^{1}	1.60E-02	0.31	38	30	moderate
night wake activity	*Oregon R* vs trp^{1}	6.70E-02	0.20	53	30	
day sleep	*iso* vs trp^{MB}	5.80E-01	0.09	16	24	
night sleep	*iso* vs trp^{MB}	3.70E-03	0.46	16	24	moderate
day sleep bout length	*iso* vs trp^{MB}	3.20E-01	0.16	16	24	
night sleep bout length	*iso* vs trp^{MB}	3.90E-01	0.14	16	24	
day wake activity	*iso* vs trp^{MB}	1.40E-03	0.49	16	24	moderate
night wake activity	*iso* vs trp^{MB}	2.90E-01	0.17	16	24	

b.ERG parameter	genotype comparison	p-value	effect size *r*	n1	n2	significant *r* magnitude
receptor potential	*iso* vs trp^{1}	7.40E-07	0.85	3*4	3*4	large
on transient	*iso* vs trp^{1}	2.20E-05	0.78	3*4	3*4	large
off transient	*iso* vs trp^{1}	7.40E-07	0.85	3*4	3*4	large
receptor potential	*iso* vs trp^{MB}	4.40E-03	0.53	3*4	4*4	large
on transient	*iso* vs trp^{MB}	2.60E-07	0.83	3*4	4*4	large
off transient	*iso* vs trp^{MB}	5.70E-01	0.11	3*4	4*4	

n1 = sample size for first genotype in genotype comparison; **n2** = sample size for first genotype in genotype comparison; * sample size in ERG statistic is actual sample size multiple with four reflecting the values from the four light intensities

Table 4. Statistics of a. sleep and b. ERG parameters for *trpl* mutants

a. sleep parameter	genotype comparison	p-value	effect size *r*	n1	n2	significant *r* magnitude
day sleep	*iso* vs $trpl^{302}$	1.40E-02	0.32	30	30	moderate
night sleep	*iso* vs $trpl^{302}$	1.40E-06	0.62	30	30	large
day sleep bout length	*iso* vs $trpl^{302}$	1.40E-06	0.59	30	30	large
night sleep bout length	*iso* vs $trpl^{302}$	6.90E-01	0.05	30	28	
day wake activity	*iso* vs $trpl^{302}$	1.10E-08	0.68	30	30	large
night wake activity	*iso* vs $trpl^{302}$	9.70E-04	0.43	30	28	moderate
day sleep	*iso/iso* vs $trpl^{302}/trpl^{MB}$	3.70E-05	0.63	23	24	large
day sleep	*iso/iso* vs $trpl^{MB}/trpl^{MB}$	3.71E-02	0.32	23	21	moderate
day sleep	$trpl^{302}/trpl^{MB}$ vs $trpl^{MB}/trpl^{MB}$	2.57E-02	0.35	24	21	moderate
night sleep	*iso/iso* vs $trpl^{302}/trpl^{MB}$	7.32E-02	0.28	24	22	
night sleep	*iso/iso* vs $trpl^{MB}/trpl^{MB}$	1.56E-02	0.39	24	23	moderate
night sleep	$trpl^{302}/trpl^{MB}$ vs $trpl^{MB}/trpl^{MB}$	1.52E-01	0.22	22	23	
day sleep bout length	*iso/iso* vs $trpl^{302}/trpl^{MB}$	1.60E-04	0.59	23	23	large
day sleep bout length	*iso/iso* vs $trpl^{MB}/trpl^{MB}$	1.60E-04	0.58	23	21	large
day sleep bout length	$trpl^{302}/trpl^{MB}$ vs $trpl^{MB}/trpl^{MB}$	8.69E-01	0.03	23	21	
night sleep bout length	*iso/iso* vs $trpl^{302}/trpl^{MB}$	2.20E-01	0.23	23	22	
night sleep bout length	*iso/iso* vs $trpl^{MB}/trpl^{MB}$	8.42E-02	0.31	23	21	
night sleep bout length	$trpl^{302}/trpl^{MB}$ vs $trpl^{MB}/trpl^{MB}$	4.82E-01	0.13	22	21	
day wake activity	*iso/iso* vs $trpl^{302}/trpl^{MB}$	1.20E-04	0.58	23	23	large
day wake activity	*iso/iso* vs $trpl^{MB}/trpl^{MB}$	9.63E-01	0.01	23	21	
day wake activity	$trpl^{302}/trpl^{MB}$ vs $trpl^{MB}/trpl^{MB}$	1.04E-03	0.50	23	21	large
night wake activity	*iso/iso* vs $trpl^{302}/trpl^{MB}$	2.91E-01	0.21	23	22	
night wake activity	*iso/iso* vs $trpl^{MB}/trpl^{MB}$	6.31E-01	0.12	23	21	
night wake activity	$trpl^{302}/trpl^{MB}$ vs $trpl^{MB}/trpl^{MB}$	7.10E-01	0.07	22	21	
b.ERG parameter	**genotype comparison**	**p-value**	**effect size *r***	**n1**	**n2**	**significant *r* magnitude**
receptor potential	*iso* vs $trpl^{302}$	2.20E-01	0.26	6*4	3*4	
receptor potential	*iso* vs $trpl^{MB}$	8.60E-01	0.03	6*4	5*4	
receptor potential	$trpl^{302}$ vs $trpl^{MB}$	2.20E-01	0.26	3*4	5*4	
on transient	*iso* vs $trpl^{302}$	2.40E-06	0.73	6*4	3*4	large
on transient	*iso* vs $trpl^{MB}$	1.50E-01	0.22	6*4	5*4	
on transient	$trpl^{302}$ vs $trpl^{MB}$	1.00E-05	0.80	3*4	5*4	large
off transient	*iso* vs $trpl^{302}$	4.80E-09	0.81	6*4	3*4	large
off transient	*iso* vs $trpl^{MB}$	9.90E-01	0.00	6*4	5*4	
off transient	$trpl^{302}$ vs $trpl^{MB}$	5.30E-08	0.81	3*4	5*4	large

n1 = sample size for first genotype in genotype comparison; **n2** = sample size for first genotype in genotype comparison; * sample size in ERG statistic is actual sample size multiple with four reflecting the values from the four light intensities

Table 4. Statistics of a. sleep and b. ERG parameters for *hdc* mutants

a. sleep parameter	genotype comparison	p-value	effect size *r*	n1	n2	significant *r* magnitude
day sleep	hdc^{MB} vs *iso*	1.10E-01	0.29	16	16	
day sleep	hdc^{JK910} vs *iso*	7.30E-05	0.60	24	23	large
night sleep	hdc^{MB} vs *iso*	1.40E-02	0.44	16	16	moderate
night sleep	hdc^{JK910} vs *iso*	4.99E-03	0.42	24	23	moderate
day sleep bout length	hdc^{MB} vs *iso*	4.50E-01	0.14	16	16	
day sleep bout length	hdc^{JK910} vs *iso*	1.08E-01	0.25	24	23	
night sleep bout length	hdc^{MB} vs *iso*	1.50E-02	0.43	16	16	moderate
night sleep bout length	hdc^{JK910} vs *iso*	2.50E-06	0.66	24	23	large
day wake activity	hdc^{MB} vs *iso*	8.70E-01	0.03	16	16	
day wake activity	hdc^{JK910} vs *iso*	2.50E-01	0.19	24	23	
night wake activity	hdc^{MB} vs *iso*	5.60E-01	0.11	16	16	
night wake activity	hdc^{JK910} vs *iso*	1.50E-06	0.68	24	23	large
b.ERG parameter	**genotype comparison**	**p-value**	**effect size *r***	**n1**	**n2**	**significant *r* magnitude**
receptor potential	*iso* vs hdc^{JK910}	8.00E-01	0.24	3*4	3*4	
receptor potential	*iso* vs hdc^{MB}	4.00E-01	0.06	3*4	3*4	
receptor potential	hdc^{JK910} vs hdc^{MB}	3.80E-01	0.32	3*4	3*4	
on transient	*iso* vs hdc^{JK910}	4.20E-05	0.72	3*4	3*4	large
on transient	*iso* vs hdc^{MB}	2.20E-04	0.79	3*4	3*4	large
on transient	hdc^{JK910} vs hdc^{MB}	9.32E-01	0.02	3*4	3*4	
off transient	*iso* vs hdc^{JK910}	5.50E-05	0.85	3*4	3*4	large
off transient	*iso* vs hdc^{MB}	2.20E-06	0.85	3*4	3*4	large
off transient	hdc^{JK910} vs hdc^{MB}	4.80E-04	0.72	3*4	3*4	large

n1 = sample size for first genotype in genotype comparison; **n2** = sample size for first genotype in genotype comparison; * sample size in ERG statistic is actual sample size multiple with four reflecting the values from the four light intensities

Table 5. Statistics of a. sleep and b. ERG parameters for *ort* mutants

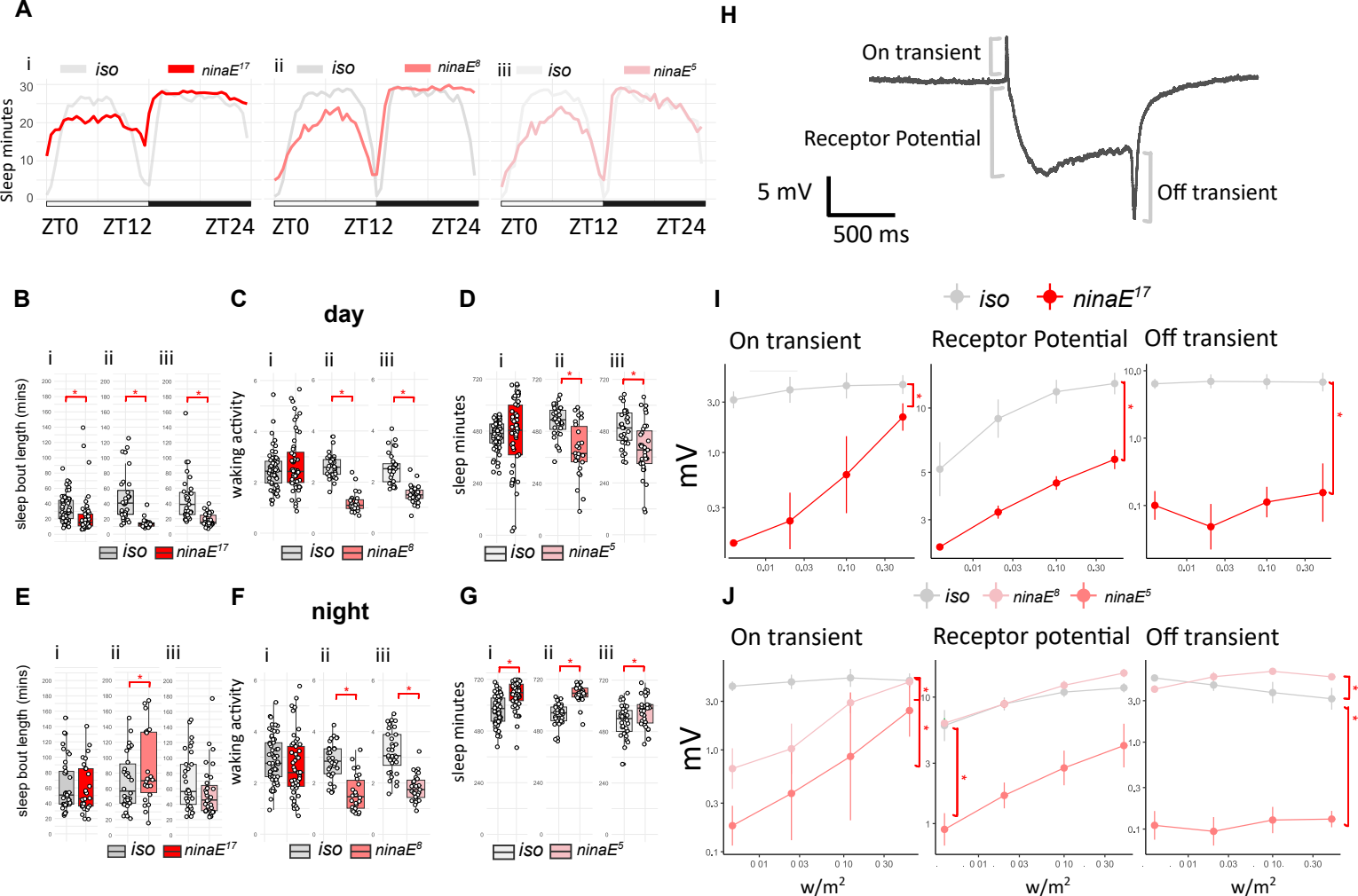
a. sleep parameter	genotype comparison	p-value	effect size *r*	n1	n2	significant *r* magnitude
day sleep	*Canton S* vs ort^{1}	5.20E-03	0.27	60	47	small
night sleep	*Canton S* vs ort^{1}	1.00E-01	0.16	60	47	
day sleep bout length	*Canton S* vs ort^{1}	2.60E-04	0.35	60	47	moderate
night sleep bout length	*Canton S* vs ort^{1}	7.30E-01	0.04	60	47	
day wake activity	*Canton S* vs ort^{1}	1.10E-08	0.55	60	47	large
night wake activity	*Canton S* vs ort^{1}	6.60E-01	0.03	60	47	
day sleep	*iso* vs ort^{attp}	4.10E-09	0.68	48	29	large
day sleep	*iso* vs $ort^{attp/+}$	8.30E-01	0.02	48	49	
day sleep	ort^{attp} vs $ort^{attp/+}$	4.50E-10	0.73	29	49	large
night sleep	*iso* vs ort^{attp}	6.10E-09	0.67	48	29	large
night sleep	*iso* vs $ort^{attp/+}$	1.50E-02	0.25	48	49	small
night sleep	ort^{attp} vs $ort^{attp/+}$	5.50E-10	0.72	29	49	large
day sleep bout length	*iso* vs ort^{attp}	6.20E-02	0.21	48	29	
day sleep bout length	*iso* vs $ort^{attp/+}$	1.80E-04	0.39	48	49	moderate
day sleep bout length	ort^{attp} vs $ort^{attp/+}$	9.00E-07	0.55	29	49	large
night sleep bout length	*iso* vs ort^{attp}	1.50E-02	0.32	48	29	moderate
night sleep bout length	*iso* vs $ort^{attp/+}$	7.50E-02	0.20	48	49	
night sleep bout length	ort^{attp} vs $ort^{attp/+}$	3.71E-01	0.10	29	49	
day wake activity	*iso* vs ort^{attp}	9.90E-07	0.55	48	29	large
day wake activity	*iso* vs $ort^{attp/+}$	3.00E-02	0.22	48	49	small
day wake activity	ort^{attp} vs $ort^{attp/+}$	6.70E-05	0.45	29	49	moderate
night wake activity	*iso* vs ort^{attp}	4.70E-08	0.60	48	29	large
night wake activity	*iso* vs $ort^{attp/+}$	4.00E-03	0.29	48	49	small
night wake activity	ort^{attp} vs $ort^{attp/+}$	3.00E-06	0.52	29	49	large
b.ERG parameter	**genotype comparison**	**p-value**	**effect size *r***	**n1**	**n2**	**significant *r* magnitude**
receptor potential	*iso* vs ort^{1}	2.70E-08	0.83	5*4	3*4	large
receptor potential	*iso* vs ort^{attp}	9.80E-01	0.01	5*4	3*4	
receptor potential	ort^{1} vs ort^{attp}	1.10E-06	0.85	3*4	3*4	large
on transient	*iso* vs ort^{1}	9.30E-08	0.81	5*4	3*4	large
on transient	*iso* vs ort^{attp}	9.30E-08	0.81	5*4	3*4	large
on transient	ort^{1} vs ort^{attp}	2.20E-01	0.26	3*4	3*4	
off transient	*iso* vs ort^{1}	2.70E-08	0.83	5*4	3*4	large
off transient	*iso* vs ort^{attp}	4.90E-06	0.83	5*4	3*4	large
off transient	ort^{1} vs ort^{attp}	7.90E-01	0.06	3*4	3*4	

n1 = sample size for first genotype in genotype comparison; **n2** = sample size for first genotype in genotype comparison; * sample size in ERG statistic is actual sample size multiple with four reflecting the values from the four light intensities

Table 6. Statistics of sleep parameters for *gmrGal4>UAS-Kir2 and Rh1Gal4>UAS-Kir2* lines

sleep parameter	genotype comparison	p-value	effect size *r*	n1	n2	significant *r* magnitude
day sleep	*gmr-Gal4>+* vs *gmr-Gal4>UAS-Kir2*	6.90E-03	0.36	32	32	moderate
day sleep	*gmr-Gal4>+* vs *+>UAS-Kir2*	1.18E-01	0.20	32	32	
day sleep	*gmr-Gal4>UAS-Kir2* vs *+>UAS-Kir2*	1.50E-04	0.51	32	32	large
night sleep	*gmr-Gal4>+* vs *gmr-Gal4>UAS-Kir2*	3.90E-01	0.11	32	32	
night sleep	*gmr-Gal4>+* vs *+>UAS-Kir2*	2.10E-06	0.62	32	32	large
night sleep	*gmr-Gal4>UAS-Kir2* vs *+>UAS-Kir2*	3.80E-04	0.46	32	32	moderate
day sleep bout length	*gmr-Gal4>+* vs *gmr-Gal4>UAS-Kir2*	8.80E-01	0.02	32	32	
day sleep bout length	*gmr-Gal4>+* vs *+>UAS-Kir2*	6.00E-01	0.11	32	32	
day sleep bout length	*gmr-Gal4>UAS-Kir2* vs *+>UAS-Kir2*	6.00E-01	0.12	32	32	
night sleep bout length	*gmr-Gal4>+* vs *gmr-Gal4>UAS-Kir2*	2.70E-01	0.14	32	32	
night sleep bout length	*gmr-Gal4>+* vs *+>UAS-Kir2*	1.14E-01	0.22	32	32	
night sleep bout length	*gmr-Gal4>UAS-Kir2* vs *+>UAS-Kir2*	5.50E-02	0.30	32	32	
day wake activity	*gmr-Gal4>+* vs *gmr-Gal4>UAS-Kir2*	9.73E-01	0.01	32	32	
day wake activity	*gmr-Gal4>+* vs *+>UAS-Kir2*	7.10E-03	0.35	32	32	moderate
day wake activity	*gmr-Gal4>UAS-Kir2* vs *+>UAS-Kir2*	7.10E-03	0.37	32	32	moderate
night wake activity	*gmr-Gal4>+* vs *gmr-Gal4>UAS-Kir2*	1.10E-04	0.50	32	32	moderate
night wake activity	*gmr-Gal4>+* vs *+>UAS-Kir2*	3.32E-02	0.29	32	32	small
night wake activity	*gmr-Gal4>UAS-Kir2* vs *+>UAS-Kir2*	2.37E-01	0.15	32	32	
day sleep	*+>UAS-Kir2* vs *Rh1-Gal4>+*	2.60E-02	0.29	28	31	small
day sleep	*+>UAS-Kir2* vs *Rh1-Gal4>UAS-Kir2*	1.10E-02	0.36	28	30	moderate
day sleep	*Rh1-Gal4>+* vs *Rh1-Gal4>UAS-Kir2*	2.00E-05	0.58	31	30	large
night sleep	*+>UAS-Kir2* vs *Rh1-Gal4>+*	4.90E-08	0.74	28	31	large
night sleep	*+>UAS-Kir2* vs *Rh1-Gal4>UAS-Kir2*	3.99E-03	0.38	28	30	moderate
night sleep	*Rh1-Gal4>+* vs *Rh1-Gal4>UAS-Kir2*	9.40E-04	0.44	31	30	moderate
day sleep bout length	*+>UAS-Kir2* vs *Rh1-Gal4>+*	8.74E-01	0.02	28	31	
day sleep bout length	*+>UAS-Kir2* vs *Rh1-Gal4>UAS-Kir2*	6.60E-03	0.37	28	30	moderate
day sleep bout length	*Rh1-Gal4>+* vs *Rh1-Gal4>UAS-Kir2*	1.50E-03	0.44	31	30	moderate
night sleep bout length	*+>UAS-Kir2* vs *Rh1-Gal4>+*	6.70E-01	0.07	28	31	
night sleep bout length	*+>UAS-Kir2* vs *Rh1-Gal4>UAS-Kir2*	4.40E-01	0.19	28	30	
night sleep bout length	*Rh1-Gal4>+* vs *Rh1-Gal4>UAS-Kir2*	6.70E-01	0.06	31	30	
day wake activity	*+>UAS-Kir2* vs *Rh1-Gal4>+*	3.10E-04	0.48	28	31	moderate
day wake activity	*+>UAS-Kir2* vs *Rh1-Gal4>UAS-Kir2*	4.40E-06	0.63	28	30	large
day wake activity	*Rh1-Gal4>+* vs *Rh1-Gal4>UAS-Kir2*	9.60E-01	0.01	31	30	
night wake activity	*+>UAS-Kir2* vs *Rh1-Gal4>+*	4.80E-07	0.62	28	31	large
night wake activity	*+>UAS-Kir2* vs *Rh1-Gal4>UAS-Kir2*	6.90E-08	0.68	28	30	large
night wake activity	*Rh1-Gal4>+* vs *Rh1-Gal4>UAS-Kir2*	2.00E-01	0.17	31	30	

n1 = sample size for first genotype in genotype comparison; **n2** = sample size for first genotype in genotype comparison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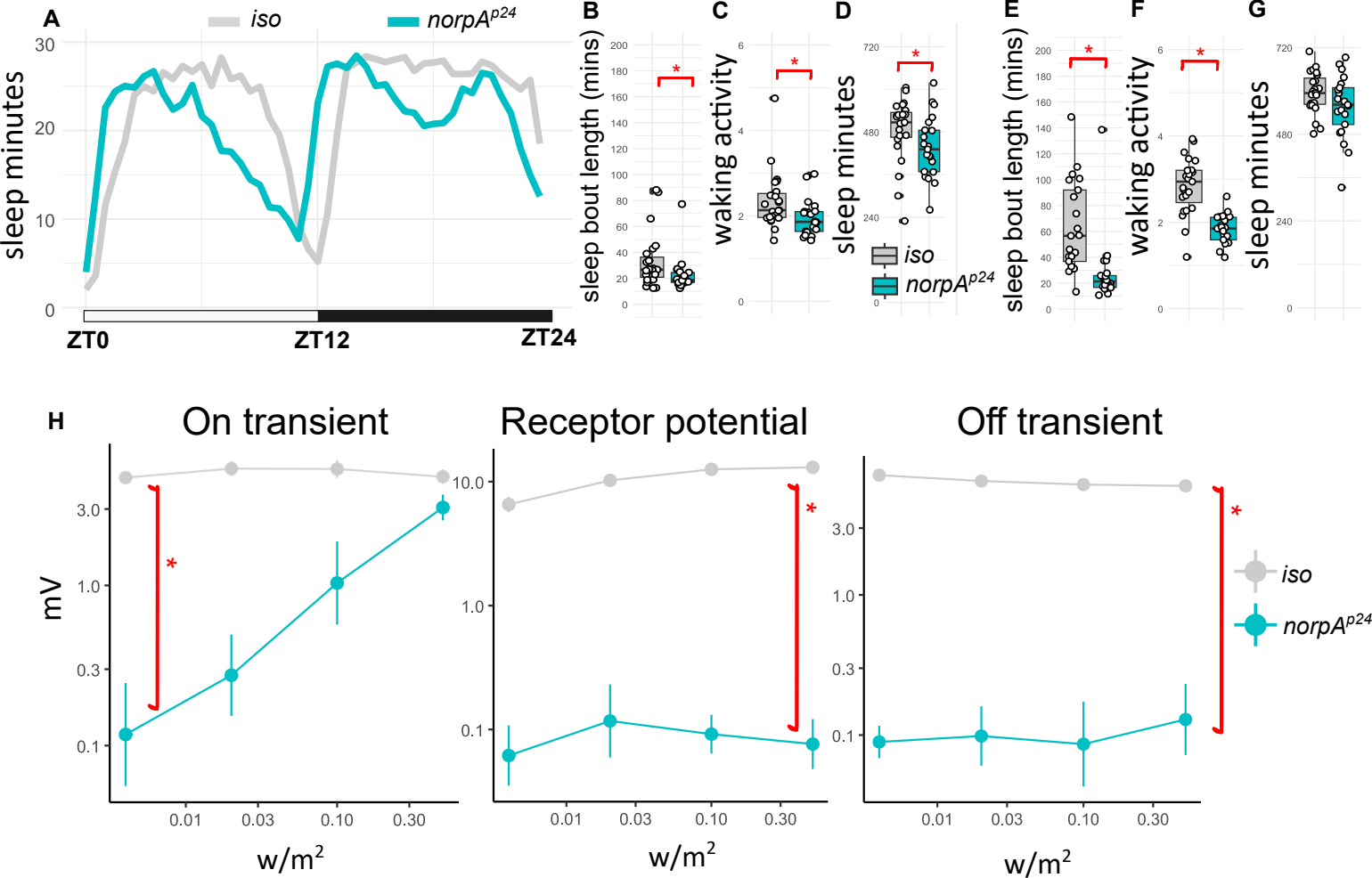
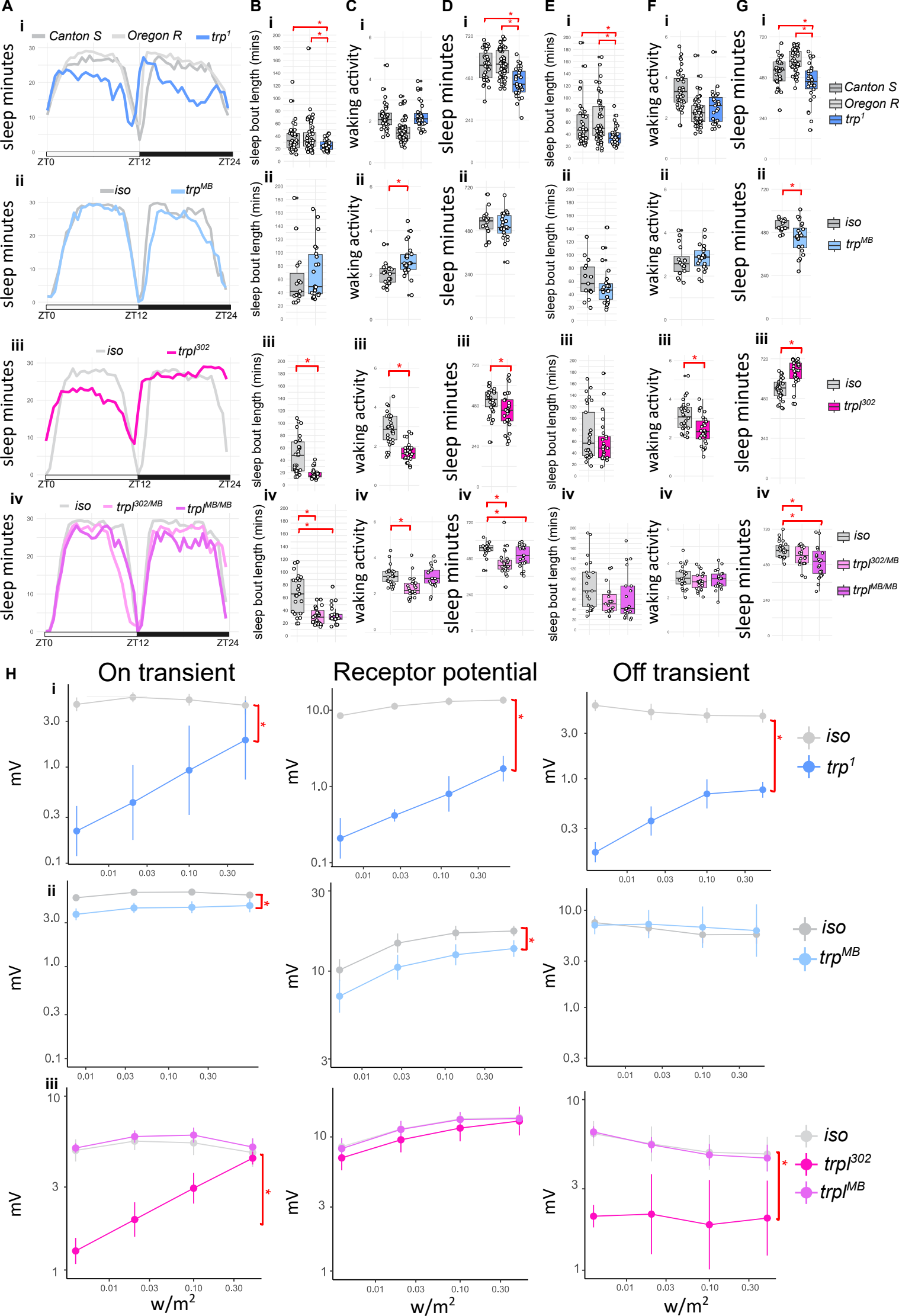
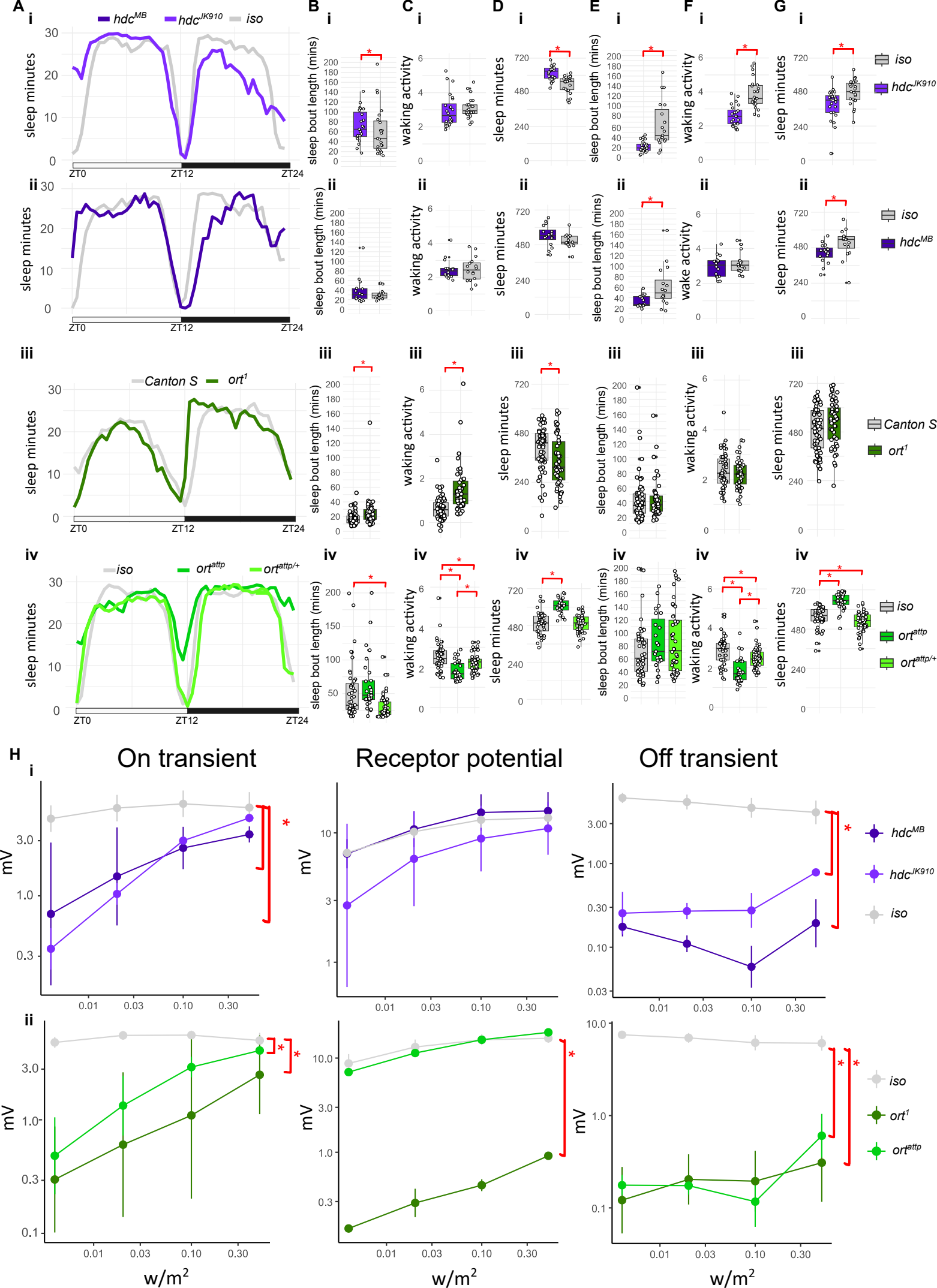
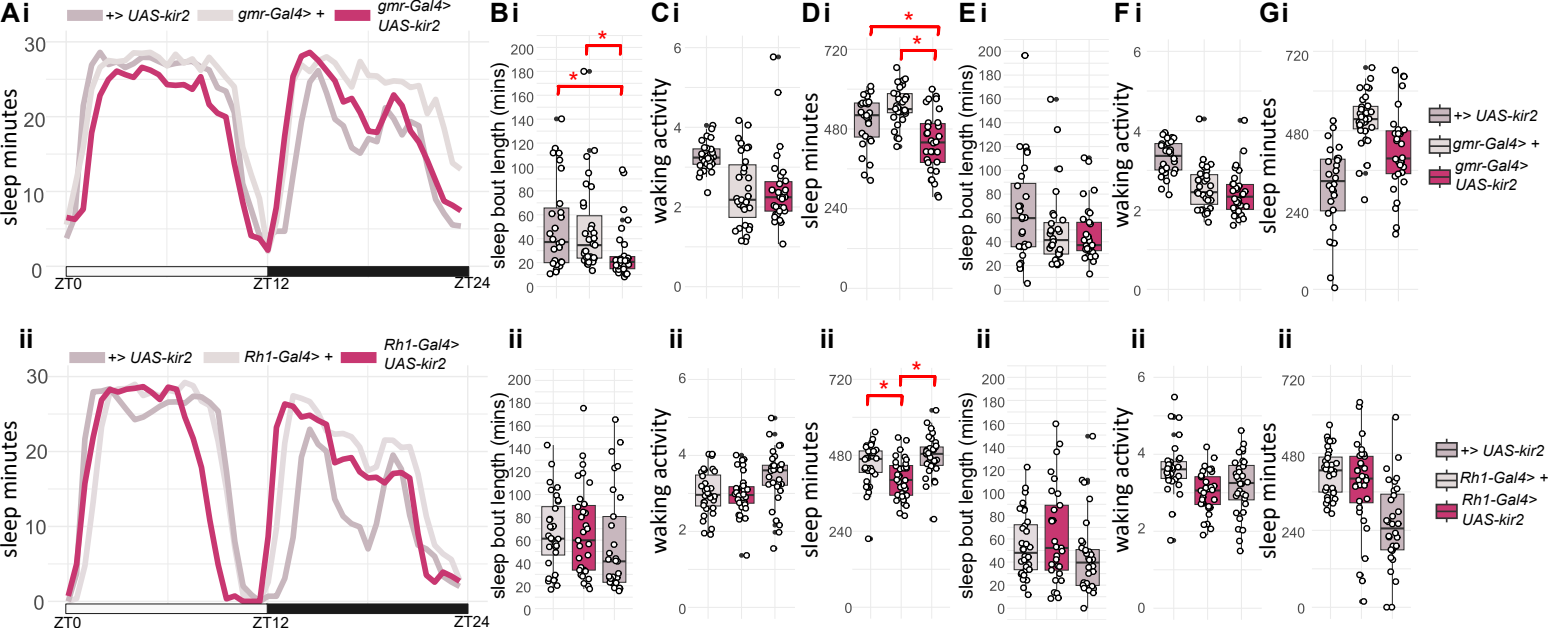
i
iso
ninaE17
ii
iso
ninaE8
iii
iso
ninaE5
Sleep minutes
30
20
10
0
ZT0
ZT12
ZT24
H
On transient
Receptor Potential
Off transient
5 mV
500 ms
B
C
day
D
sleep bout length (mins)
waking activity
sleep minutes
iso
ninaE17
ninaE8
ninaE5
E
F
night
G
I
iso
ninaE17
On transient
Receptor Potential
Off transient
mV
J
iso
ninaE8
ninaE5
Receptor potential
w/m2


A
iso
norpA^p24
sleep minutes
30
20
10
0
ZT0
ZT12
ZT24
B
sleep bout length (mins)
C
waking activity
D
sleep minutes
iso
norpA^p24
E
sleep bout length (mins)
F
waking activity
G
sleep minutes
H
On transient
Receptor potential
Off transient
mV
w/m2
0.01
0.03
0.10
0.30
iso
norpA^p24


A
B
C
D
E
F
G
H
i
ii
iii
iv
Canton S
Oregon R
trp1
iso
trpMB
trpl302
trpl302/MB
trplMB/MB
sleep minutes
sleep bout length (mins)
waking activity
ZT0
ZT12
ZT24
On transient
Receptor potential
Off transient
mV
w/m2
0.01
0.03
0.10
0.30


A
B
C
D
E
F
G
H
i
ii
iii
iv
hdc^MB
hdc^JK910
iso
Canton S
ort^1
ort^attp
ort^attp/+
sleep minutes
sleep bout length (mins)
waking activity
wake activity
ZT0
ZT12
ZT24
On transient
Receptor potential
Off transient
mV
w/m^2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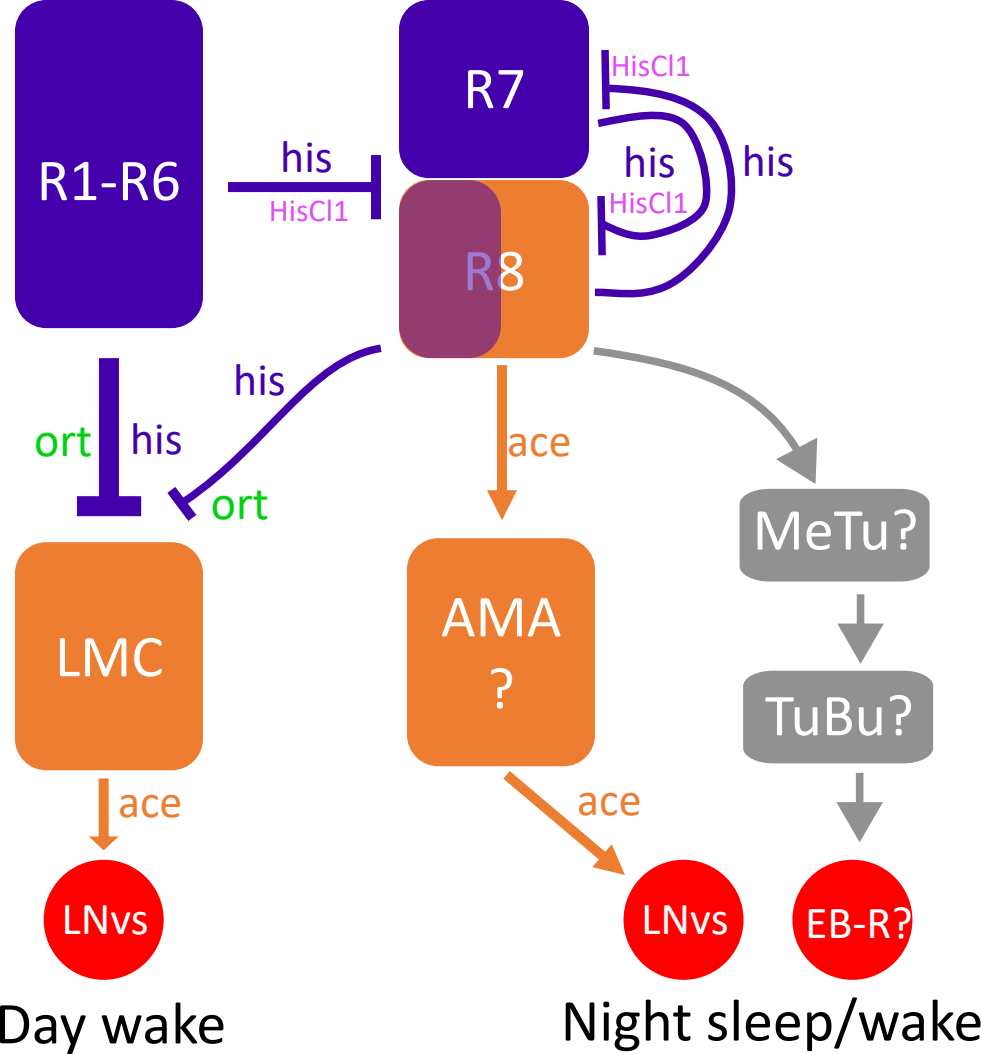
+> UAS-kir2
gmr-Gal4> +
gmr-Gal4> UAS-kir2
sleep minutes
ZT0
ZT12
ZT24
Bi
sleep bout length (mins)
Ci
waking activity
Di
sleep minutes
Ei
sleep bout length (mins)
Fi
waking activity
Gi
sleep minutes
+> UAS-kir2
gmr-Gal4> +
gmr-Gal4> UAS-kir2
ii
+> UAS-kir2
Rh1-Gal4> +
Rh1-Gal4> UAS-kir2
sleep minutes
ZT0
ZT12
ZT24
sleep bout length (mins)
waking activity
sleep minutes
sleep bout length (mins)
waking activity
sleep minutes
+> UAS-kir2
Rh1-Gal4> +
Rh1-Gal4> UAS-kir2


R1-R6
his
HisCl1
R7
HisCl1
his
his
HisCl1
R8
his
ort
his
ort
ace
MeTu?
LMC
AMA
?
TuBu?
ace
ace
LNvs
LNvs
EB-R?
Day wake
Night sleep/wake