A sleep promoting role of phototransduction in *Drosophila melanogaster*

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Abstract:

Daily sleep-wake cycle is a conserved behaviour defined by locomotion quiescence and enhanced responsive threshold to sensory stimuli. Both circadian rhythm and a homeostatic process determine the daily sleep profile, which is also regulated by environmental light, a major sensory input to regulate circadian rhythm and alertness. With decades of investigation in *Drosophila*, the cellular and circuital mechanism 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 have started an investigatory survey of sleep behaviour using classic mutant lines to manipulate phototransduction and downstream neural transmission. We observed consistent day sleep fragmentation in flies with mutations in multiple phototransduction components. We also found hyperpolarised *Drosophila* photoreceptor 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. In summary, we provide a rigorous quantification of several phototransduction genes and reveal the key role of visual input to promote sleep.

1. Introduction

Sleep is vital for health. Disruptions in timing and/or amount of sleep has been linked to various metabolic and neurological diseases. Across 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 to 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 flies *Drosophila* has been used for identifying the biological basis and the conserved function of sleep for the past two decades: In parallel to murine investigations, studies in *Drosophila* have now 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* also show 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).

The onset and the duration of daily sleep are regulated by two intrinsic mechanisms: the circadian rhythm and homeostasis. The circadian rhythm defines the baseline sleep-wake level across 24 hours, whereas the homeostatic process maintains the overall level of sleep amount. In *Drosophila*, the neural circuits that generate circadian rhythm reside in the 242 so-called clock neurons (Reinhard et al., 2024) that express the core molecular clock mechanism, whereas the homeostatic control of sleep are distributed among neural domains including Fan-Shaped Body (FSB) and Ellipsoid Body (EB) (Jones et al., 2025; Shafer and Keene, 2021). The two mechanisms are likely coordinated as a recent study also demonstrated that clock neurons modulate the FSB-mediated sleep homeostat (Hsu et al., 2025).

Light is a prominent environmental stimulus to regulate both mechanisms in order to maintain animal's temporal niches with sufficient sleep. Such synchronisation between environmental light regime and the neural activity of clock neurons is called circadian photoentrainment. Similar to the mammalian system, visual inputs are part of photoentrainment in *Drosophila*. Visual system in the fly is comprise of peripheral light sensing neurons (photoreceptors in compound eyes, HB-eyelet and ocelli) and their downstream visual processing brain areas called the optic lobes and ventrolateral neuropils (Nériec and Desplan, 2016). Light signal is converted into membrane electrical depolarisation in the photoreceptors in compound eye and HB-eyelet by activation of cation channel

57 triggered by the *Rhodopsin* 1-6 (*Rh1-6*) linked phototransduction/GPCR signal cascades (Hardie,
58 2012). Activation of these two types of light-sensing neurons then release histamine or acetylcholine
59 to communicate with clock neurons either directly or indirectly via neural network in optic lobes
60 (Alejevski et al., 2019; Ogueta et al., 2020, 2018; Rieger et al., 2003; Schlichting et al., 2016; Tang et
61 al., 2022; Xiao et al., 2023). Apart from visual system, *Drosophila* molecular clock can also be
62 entrained by the cell autonomous light-sensing mechanisms mediated by *cryptochrome* (*cry*),
63 *quasimodo* (*qsm*) or *Rhodopsin 7* (*Rh7*) in clock neurons (Chen et al., 2011; Ni et al., 2017; Peschel et
64 al., 2009).

65 Light can directly regulate sleep by either promoting or supressing sleep via different neural
66 pathways in the murine system (Altimus et al., 2008; Pilorz et al., 2016). As a crepuscular species,
67 *Drosophila* sleep both in the day with the light and in the night in the darkness. While current
68 evidence indicate that distinct neurogenetic network may control day and night sleep (Chen et al.,
69 2019; Damulewicz et al., 2020; Hsu et al., 2025; Ishimoto et al., 2012; Lamaze et al., 2018; Sengupta
70 et al., 2019), visual experience in the light was recently found to promote sleep in *Drosophila*
71 (Kirszenblat et al., 2019), suggesting light and/or vision per se can serve as sleep homeostat drives,
72 similar to vertebrates (Horne and Walmsley, 1976; Korf et al., 2017; Miyamoto et al., 2003).

73 Despite growing research interests (Mazzotta et al., 2020), the mechanism underlying light-mediated
74 regulation of *Drosophila* sleep homeostat is far from clear: a previous study showed that synaptic
75 inhibition in *Drosophila* eye photoreceptor promote sleep (Damulewicz et al., 2020), yet the
76 optogenetic activation of all eye photoreceptors in another investigation did not result in sleep
77 suppression (Kirszenblat et al., 2019). Moreover, artificial activation of the direct downstream target
78 of eye photoreceptor, lamina monopolar neuron 2 (L2 LMC), resulted in drastic sleep loss (Muraro
79 and Ceriani, 2015). This finding is in contradiction to the increased sleep observed in flies with
80 reduced histamine (Oh et al., 2013), which is the main neurotransmitters released from
81 photoreceptors to inhibit L2 LMC neurons (Pantazis et al., 2008a). A recent systematic study of
82 mutants with defects in various light input pathway indicates that only *Rh7* is required for shade
83 sleep preference in the day, but the study did not investigate the baseline sleep level in these
84 mutants (Meyerhof et al., 2024).

85 In this study, we investigated the role of light input pathways in *Drosophila* sleep by focusing on the
86 role of visual system. Using the conventional infrared beam-based sleep assay (Chen et al., 2019;
87 Chiu et al., 2010), we surveyed the baseline sleep profiles for an array of fly lines with mutations in
88 genes involved in a) phototransduction, and the downstream histaminergic neurotransmission.
89 (Hardie, 2012) as well as hyperpolarising photoreceptors. Unlike night sleep, day sleep in *Drosophila*

is highly modifiable by sex, mating and nutrient status (Dove et al., 2017; Duhart et al., 2023; Isaac et al., 2009; Wu et al., 2018). Typically, mated females can sleep as little as 150-200 minutes as compare to 300-400 minutes in males in the 720 minutes of day time. In order to capture both sleep increase and decrease upon genetic manipulation, we focused our investigation in virgin male flies. By carefully examining sleep parameters and the phototransduction/neurotransmission defects in electroretinogram (ERG), we identified a novel role of visual phototransduction in driving sleep.

2. Materials and methods

2.1 Fly husbandry

Drosophila strains are kept in the standard fly food (glucose 6.93% w/v, agar 0.5% w/v, cornmeal, 6.3% 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. The long-term stocks are kept in the same food and light regime at 18°C. The Canton S and Oregon R wildtype strains are obtained from Kyriacou Lab (University of Leicester, UK), while w^{iso31} (i.e. *iso*) control is obtained from Jepson lab (University College London, UK). Following visual mutants, Gal4 and UAS transgenic are ordered from Bloomington stock centres (stock number): trp^{1} (5692); trp^{MB} (23636); $trpl^{302}$ (31433); $trpl^{MB}$ (29134); $ninaE^{8}$ (2001); $ninaE^{5}$ (3531); $ninaE^{17}$ (5701); $norpA^{P24}$ (9048); hdc^{MB} (25260); hdc^{JK910} (64203); ort^{1} (1133); ort^{attP} (84559); *Rh1-Gal4* (8691); *gmr-Gal4* (1104); *UAS-kir2* (6595); *UAS-TeTxLC* (28837); *UAS-myr-mRFP* (7118). The 1st and 3rd chromosomes of hdc^{JK910} were replace with those from w^{iso31} stock via following balancer crossings with w^{iso31C}*;Sco/CyO; MKRS/TM6B* and w^{iso31C}*;Sco/CyO; +/+* (gifts from Jepson lab). All Gal4 and UAS transgenic are brough into w^{iso31} background via five generation outcrossing following *[w+]* eye colour marker cassette. For *Rh1-Gal4* outcrossing, we use *UAS-myr-mRFP/+; Rh1-Gal4/+* and follows the eye RFP signal as visible marker under fluorescent scope for each generation. The final stock are then established by balancer crossing with w^{iso31C}*;Sco/CyO; MKRS/TM6B and then* w^{iso31C}*;+/+; MKRS/TM6B* for removing UAS-myr-mRFP.

2.2 Sleep recording and analysis

Prior sleep experiment, *Drosophila* strain or crossings are reared in a richer food 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% w/v, cornmeal 1.5% w/v, yeast 3.5% w/v, propionic acid 0.5% v/v, nipagin 0.1% v/v), as to keep the rearing condition consistent with previous experimental conditions (Chen et al., 2019, 2011; Lamaze et al., 2018). Also, we notice flies are generally less reproductively successful and motile in standard food. The sleep recording are performed as described in (Chen et al., 2019): three to four days old virgin adult male are transferred to typical behaviour tubes containing food (2% w/v agar and 4% w/v sucrose at one

122 end and cotton at the other end), and housed in typical Drosophila Activity Monitor (DAM,
123 Trikinetics Inc.) for 3 days of 12hours light-dark cycle at 25°C in a temperature and light controlled
124 incubator (MIR-254-PE, PHCbi). The lighting source is a day light fluorescent lamp (FL15D, 6500K)
125 with intensity between 1.2 and 6.7 w/m^2 depending on the distance to the lamp. The locomotion of
126 each fly is recorded as infrared beam crossing per minutes. The locomotion data for day 3 are
127 outputted into a previously customised Excel Calculator (Chen et al., 2019) to calculate all sleep
128 episodes (any period of immobility ≥ 5 minutes) along 24 hours and generate the following
129 parameters: **overall sleep profile**: minutes of sleep per 30 mins across 24 hours; **day sleep**: total
130 minutes in sleep during 12 hours of day; **night sleep**: total minutes in sleep during 12 hours of night;
131 **day average sleep bout length**: day sleep minutes divided by total numbers of sleep bout during 12
132 hours of day; **night average sleep bout length:** night sleep minutes divided by total numbers of sleep
133 bout during 12 hours of night; **day wake activity**: number of beam crosses divided by total wake
134 minutes during 12 hours of day**; night wake activity**: number of beam crosses divided by total wake
135 minutes during 12 hours of night.

136 **2.3 Electroretinogram (ERG)**

137 Three-to-five days old adult virgin male flies were inserted into a p1000 plastic pipette tip, so that its
138 head protruded from the narrow ending. The head was fixed using melted honey wax applied
139 between the thorax and head without blocking the mouthpart and eyes. The fly mounted pipette tip
140 was kept in the dark for 20 mins and then transferred into the ERG recording rig. Glass electrodes
141 (Borosilicate) with resistance of ~5 MO and filled with 3 M KCl were inserted into the compound eye
142 (recording) and head (reference). Each fly was then rest in the dark for 3 minutes before exposed to
143 four light intensities: 0.004, 0.02, 0.1 and 0.5 w/m^2 at 540nm LED green light source (M530L4-C1,
144 ThorLab). These intensities reside in the liner range of receptor potential response. We stimulated
145 with 1000 ms exposure to each light intensity and repeated two further times to get an average of
146 three presentations at each light intensity for each fly. The potential difference between the
147 electrodes was amplified 10x, and acquired by Neuroprobe Amplifier model 1600 and 8-channel
148 data acquisition interface DA-8 (A-M systems) using the SciWorks Data Acquisition & Analysis Suite.

149 **2.4 Data display and statistics**

150 `ggplot2` package in R is used to display sleep and ERG data. X-Y plots are used to display overall
151 sleep profile for each comparing genotypes. Boxplots with individual values are used to display sleep
152 parameters data. We calculated the average voltage changes of on transient, receptor potential and
153 off transient in the ERG profiles of each replicate for each fly (Figure 1H). The value for each fly is
154 then plotted against light intensity on logarithmic scales by `ggplot2`. Considering the variability in

155 sleep data (Chen et al., 2019; Lamaze et al., 2018), statistical significance are identified by Kruskal-
156 Wallis rank sum test for non-parametric multiple comparison (`kruskal.test`). Pairwise Wilcoxon
157 rank sum exact test with multiple comparison Benjamini-Hochberg correction
158 (`pairwise.wilcox.test`) is used to significant difference between pairwise genotype. ERG
159 voltage changes data are pooled among four intensities and used to identify genotypical difference
160 via Pairwise Wilcoxon rank sum exact test. Significance level is set at adj.p<0.05. Effect size *r* is
161 calculated by `wilcox_effsize` with magnitude categories: small; moderate and large assigned. All
162 statistic comparisons, p values, effect size and sample size are listed in Table1 to Table 6 for the
163 corresponding Figure 1 to Figure 5.

164 **3. Result**

165 **3.1 Defects in phototransduction genes caused short day sleep bout**

166 To verify the role of visual phototransduction genes in sleep regulation, we selected eight mutant
167 strains: *Rhodopsin 1/ninaE*, *Phospholipase C/norpA*, and the two cation channels *trp* and t*rpl*. We
168 included both classic point mutations as well as more recently generated and characterised
169 mutations using P-element insertion when available. To analyse the details of sleep-wake profiles
170 under typical day of 12 hours light-12 hours dark, we examine the three conventional parameters for
171 both day and night period: total sleep minutes, average sleep bout minutes and the waking activity.

172 **3.1.1 *ninaE***

173 Upon exposure to light, 11-cis 3-hydroxy-retinal, the chromophore in the Rhodopsin protein
174 undergoes photoisomerization, transforming Rhodopsin to Metarhodopsin and then trigger
175 downstream GPCR signal cascade. Rhodopsin 1/ninaE protein is the main light-sensing GPCR in the
176 outer photoreceptor R1-R6 of *Drosophila* compound eye. Reduction or loss of ninaE abolish or
177 reduce block photoisomerization and therefore light induced photoreceptor potential and transients
178 that is typically measurable for R1-R6 in ERG (Hardie, 2012).

179 Large part of visual tasks including navigation, phototaxis, optomotor and escapes required R1-R6
180 activity. We therefore first tested sleep profile of the class null allele $ninaE^{17}$ (O'Tousa et al., 1985).
181 During the day, $ninaE^{17}$ mutants shows lower midday sleep peak in the sleep profile (Figure 1A) but
182 the similar amount of total sleep minutes is found as compared to the white eye control, w^{iso31} (here
183 after *iso*, Figure 1Di). However, we found that the average sleep bout minutes in $ninaE^{17}$ is shorter
184 than control, indicating a sleep fragmentation (Figure 1Bi). During the night, we observed an
185 increased sleep amount (Figure 1Gi) while the average sleep bout minute is similar to control (Figure
186 1Ei). Since the sleep measurement in *Drosophila* depends on locomotion, the sleep increase can be
187 falsely claimed by defect in locomotion. Therefore "waking activity", i.e., locomotor activity per
188 waking minutes, was designed as a conventional measure to detangle such compound scenario
189 (Andretic and Shaw, 2005; Harbison and Sehgal, 2008). We applied this measurement and found
190 that waking activity are similar between $ninaE^{17}$ and control in both day and night (Figure 1Ci,Fi).

191 $ninaE^{17}$ flies contain an secondary $ebony^{1}$ mutation that impacts sleep-wake cycles (Rossi et al.,
192 2015; Suh and Jackson, 2007). We therefore tested another two *ninaE* hypomorphs $ninaE^{5}$ and
193 $ninaE^{8}$ (Scavarda et al., 1983). Both mutants show similar midday sleep peak reduction as seen in
194 $ninaE^{17}$ flies (Figure 1A) This observation is consistent with significant reduced day sleep minutes in
195 either mutant as compared to controls (Figure 1Dii-iii). Similarly, both mutants also show shorter

196 average sleep bout minutes in the day (Figure 1Bii-iii). Intriguingly, the loss of sleep in the two *ninaE*
197 hypomorphs coincide with loss of wake activity in the day (Figure 1Cii-iii). During the night, *ninaE*
198 hypomorphs both shows increased sleep (Figure 1Gii-iii) but only $ninaE^{8}$ mutant shows significant
199 increase of the average sleep bout minutes as compared to control (Figure 1Eii-iii). However,
200 mutants severely reduce their wake activity (Figure 1Fii-iii with effect size *r* between 0.67 and 0.82,
201 Table 1). Therefore, the increased night sleep may simply reflect loss of locomotor activity. To
202 confirm *ninaE* mutants harbour phototransduction defects, we measured light-evoked receptor
203 potentials through Electroretinograms (ERG, Figure 1H). Consistent with previous reports, $ninaE^{17}$
204 and $ninaE^{8}$ exhibit reduced light induction of receptor potential, on and off transients (Figure 1I-J)
205 whereas $ninaE^{5}$ retain certain function (see normal receptor potential and off-transient in $ninaE^{5}$,
206 Figure 1J). Take results from the three mutants together, we conclude the reduction or loss of
207 *Drosophila* Rhodopsin 1 (ninaE) protein result in consist reduced day sleep bout length. This finding
208 indicates that phototransduction is required to drive day sleep pressure. However, recent studies
209 have found a non-visual function of ninaE (eg, Zanini et al., 2018). To verify the shorter day sleep
210 bouts is linked to phototransduction or just pleotropic function of ninaE protien, we further
211 examined the roles in sleep for three other proteins key for phototransduction.

212 **3.1.2 *norpA***

213 Metarhodopsin triggers Gqα activation and enzymatic reaction of Phospholipase C (PLC) that
214 produce secondary messenger. norpA protein is the main PLC in the visual system, and the classic
215 mutant $norpA^{p24}$ exhibit severe visual defects (Gao et al., 2008). The sleep profile of $norpA^{p24}$ showed
216 decline of day sleep from the midday and a secondary dip in the midnight as compared to controls
217 (Figure 2A). The changes in sleep profile coincide with reduced sleep in the day (Figure 2D) and
218 shorter bout minutes in both day and night (Figure 2B, 2E). Similar to $ninaE^{5}$ and $ninaE^{8}$, $norpA^{p24}$
219 mutant have low wake activity (Figure 2C, 2F, effect size 0.39/0.75, Table 2). We also confirm
220 abnormal ERG profiles in $norpA^{p24}$ (Figure 2H).

221 **3.1.3 *trp and trpl***

222 Activation of the two ion channels trp and trpl in the last step of phototransduction cause cation
223 influx resulting in depolarisation of photoreceptor. We therefore examine the sleep profile of four
224 mutant lines, two P-element insertion mediated mutants: trp^{MB}, $trpl^{MB}$ (Chen et al., 2015) and two
225 classic null mutants: trp^{1}, and $trpl^{302}$ (Cosens and Manning, 1969; Leung et al., 2000; Niemeyer et al.,
226 1996). Except for trp^{1}, these lines are white eyes as *ninaE* and *norpA* mutants as described above.

227 *trp*^{1} mutant were first isolated from red eye wild type but the precise background is not known
228 (Cosens and Manning, 1969), so we compare its sleep profile to two widely used wild type in sleep
229 and vision research: *Canton S* and *Oregon R.* Significant reduction in day and night sleep amount and
230 average sleep bout length are shown as compared to either wild type flies (Figure 3Ai, 3Bi and 3Ei,
231 effect size: 0.29-0.54, Table 3). The wake activity of *trp*^{1} is comparable to *Canton S* but higher than
232 *Oregon R*, and has limited impact to sleep loss (Figure 3Ci and 3Fi). The sleep profile of *trp*^{MB} is
233 distinct from *trp*^{1}*, norpA* or *ninaE* mutants, with day sleep level similar to control but a significant
234 night sleep loss (Figure 3Aii, 3Gii). There are no significant changes in sleep bout minutes detected in
235 *trp*^{MB} flies (Figure 3Aii and 3Eii). Although, *trp*^{MB} flies sleep a similar amount during the day,
236 compared to control, their wake activity is significantly higher (Figure 3Cii).

237 This relative normal sleep-wake profile may relate to the less severe reduction in receptor potential
238 and light on/off transients in the ERG of *trp*^{MB} as compared to those of the *trp*^{1} null mutant (Figure
239 3Hi and 3Hii, effect size 0.11-0.83 vs 0.78-0.85, Table 3).

240 On the other hand, both *trpl*^{302} and *trpl*^{MB} mutants exhibit day sleep profile similar to *ninaE* or *norpA*
241 mutants (Figure 3Aiii and 3Aiv), with significant reduced day sleep amount and average bout
242 minutes (Figure 3Biii, 3Biv, 3Diii and 3Div). Night sleep profiles and wake activities for the two *trpl*
243 mutants diverged: *trpl*^{302} sleep more whereas *trpl*^{MB} sleep less (Figure 3Giii, 3Giv). No significant
244 changes in night sleep bout length were observed in both mutants as compared to the controls
245 (Figure 3Eiii and 3Eiv). *trpl*^{302} mutants generally have low wake activity whereas *trpl*^{MB} maintain
246 relative normal wake locomotion as compared to the controls (Figure 3Ciii, 3Civ, 3Fiii and 3Fiv).
247 *trpl*^{302} mutant is in *cn* and *bw* mutation background, which may affect the sleep. We therefore tested
248 *w; trpl*$^{302/MB}$ flies to reduce the impact of *cn bw* mutation. Similar to *trpl*^{302} and *trpl*^{MB}, the *w;*
249 *trpl*$^{302/MB}$ transheterozygote mutant showed shorter day sleep and sleep bout length (Figure 3Biv). As
250 seen in previous reports (Chen et al., 2015; Leung et al., 2000) both mutants have limited impact on
251 ERG profile, *trpl*^{302} showed large reduction of on/off transient (effect size 0.73/0.81, Table 4),
252 whereas *trpl*^{MB} maintain a normal ERG, possibly due to synaptic compensatory mechanism (Figure
253 3Hiii). Nevertheless, our data indicate the reduced trpl function and expression level cause sleep loss
254 in the day.

255 **3.2 Divergent sleep function in histaminergic pathway**

256 *Drosophila* photoreceptors can be divided into Rh1-expressing (R1-R6) and non-Rh1-exppressing
257 neurons (R7/R8). The R1-R6 photoreceptors form synapses onto lamina monopolar neurons (LMC),
258 while the R7/R8 send their output to medulla in the optic lobes. Histamine is the primary
259 neurotransmitters for Rh1-expessing photoreceptor. Therefore, we test if mutants with defects in

260 histamine synthesis or postsynaptic reception show altered sleep. Histidine decarboxylase (*hdc*) is
261 the main histamine synthesis enzyme (Pantazis et al., 2008b). We investigated the sleep profiles for
262 the two *hdc* mutants (hdc^{JK910} and hdc^{MB}) that were characterised previously and showed reduced
263 histamine levels in the photoreceptors (Burg et al., 1993; Stenesen et al., 2015).

264 As expected, both mutants selectively reduce on/off transients' levels, the components in ERG that
265 reflect synaptic transmission between photoreceptors and LMCs (Figure 4Hi). However, the sleep
266 profiles between the two mutants diverged: hdc^{JK910} showed increased day sleep and bout length,
267 whereas hdc^{MB} showed decreased day sleep and bout length (Figure 4Bi and 4Di). The wake activities
268 in the day are similar between *hdc* mutants and controls (Figure 4Ci). During the night, both *hdc*
269 mutants sleep less with shorter sleep bouts (Figure 4Ei and 4Gi). Intriguingly, both *hdc* mutants have
270 reduced wake activities compared to the controls at night (Figure 4Fi). Two histamine receptors ort
271 and HisCl1 are detected in *Drosophila* CNS, and they are both histamine gated chloride channels
272 (Pantazis et al., 2008b). Transcriptomic data in *Drosophila* visual system suggest that ort express
273 selectively in neurons postsynaptic to photoreceptors, whereas HisCl1 express almost exclusively in
274 photoreceptors as auto-receptors (Davis et al., 2020a). We therefore verify the sleep profile in ort
275 mutants to investigate the role of histamine signal downstream to photoreceptors.

276 First we monitored sleep profile of the classic ort^{1} mutant against *Canton S* as they share the same
277 genetic backgrounds (Inoue et al., 1989). A small but significant day time sleep loss are detected in
278 ort^{1} flies (Figure 4Dii, effective size 0.27, Table 5). ort^{1} sleep for longer bouts during the day but have
279 higher day wake activity compared to *Canton S* (Figure 4Bii and 4Cii). In contrast to *hdc* mutants, no
280 night sleep phenotype is detected in ort^{1} (Figure 4Eii-4Gii). We also tested a recent CRISPR-
281 mediated null mutation ort^{attp} in the white eye background (Deng et al., 2019). The sleep profile of
282 ort^{attp} is distinct from ort^{1}, showing more sleep as compared to control (Figure 4Diii and 4Giii).
283 However, the wake activities of ort^{attp} are severely reduced (Figure 4Ciii and 4Fiii, effective size
284 0.55/0.60, Table 5) potentially compounding the observed sleep phenotype. Intriguingly, shorter day
285 sleep bouts and small reduction of night sleep are detected in $ort^{attp/+}$ heterozygotes (Figure 4Biii and
286 4Giii), accompany improved wake activity (Figure 4Ciii and 4Fiii, wake activity ort^{attp} vs $ort^{attp/+}$, Table
287 5).

288 Both *ort* mutants exhibit ERG profile with loss of on/off transients, while ort^{1} also showed loss of
289 receptor potential (Figure 4Hii).

290 **3.3. Hyperpolarisation of Rh1-expressing photoreceptor causes short day sleep bout**

291 Our finding so far indicates that seven of the eight examined phototransduction mutants exhibit day
292 time sleep defects. However, there is divergence between sleep and ERG phenotypes in *trpl* and *trp*
293 mutants. Moreover, altered histaminergic pathway show divergent sleep defect in day or night.
294 Thus, we further test if direct inhibition of photoreceptor excitability causes sleep defects. At first,
295 we applied Gal-UAS system to block synaptic transmission from all photoreceptors by expressing
296 UAS-TeTxLC (tetanus toxin light chain) with gmr-Gal4 as in (Damulewicz et al., 2020). However, we
297 were not able to obtain viable adult flies even after maintaining the culture in 22°C to reduce the
298 known overexpression-related toxicity (Rezával et al., 2007; Sweeney et al., 1995). We next used
299 UAS-Kir2.1 (Baines et al., 2001; Nitabach et al., 2002) to hyperpolarise all or just Rh1-expressing
300 photoreceptors by gmr-Gal4 or Rh1-Gal4. Consistent with the finding for *ninaE* and *norpA* mutants,
301 *gmr-Gal4>uas-Kir2.1* and *Rh1-Gal4>uas-Kir2.1* flies showed reduced day sleep as compared to
302 *Gal4>+* or *+>uas-Kir2.1* controls (Figure 5Ai-ii, 5Di-ii), but only *Rh1-Gal4>uas-Kir2.1* flies exhibit
303 reduced sleep bout minutes in the day (Figure 5Bi-ii). No obvious changes in the analysed night sleep
304 parameters in both *gmr-Gal4>uas-Kir2.1* and *Rh1-Gal4>uas-Kir2.1* flies as compared to the controls
305 (Figure 5E-G). This is partly because the unexpected low sleep exhibited in *+>uas-Kir2.1* controls
306 (*gmr-Gal4>+* vs *+>uas-Kir2.1* and *Rh1-Gal4>+* vs *+>uas-Kir2.1*; effect size 0.62/0.74, Table 6). Kir2.1
307 expressing flies had no changes in wake activity (Figure 5C and 5F). Taken together, our data
308 indicates that phototransduction and the neuronal excitability in the *Drosophila* Rh1-expressing
309 photoreceptor promote day sleep.

310 **4. Discussion**

311 In this study, we surveyed ten mutants with either hyperpolarised photoreceptors or defects in
312 phototransduction, and four mutants with disrupting histaminergic transmission, the main
313 neurotransmitter used by *Drosophila photoreceptors*. We observed short- or fragmented-day sleep
314 in majority of the phototransduction mutants as well as in mutants with hyperpolarised R1-R6
315 photoreceptors. This data indicates that R1-R6 photoreceptor neural activity is required for sleep
316 homeostatic drive during daytime. In contrast, we found that reduced histamine production (*hdc*
317 mutants) result in night time sleep loss, while reduced histaminergic reception by ort mutation cause
318 short day sleep. Divergent sleep phenotypes between *hdc* and *ort* mutants may reflect a potential
319 demarcation of day and night sleep neural network downstream of photoreceptors (see below).
320 Moreover, *hdc* and *ort* expressions are detected outside photoreceptor or visual system, so we
321 cannot rule out the sleep defect we observed is exclusively derived from visual system. Importantly,
322 the mutants tested in this study don't loss their ability to sense light, as the non-visual pathway such
323 as *cry*, *Rh7* and *qsm* remain intake. Nevertheless, we showed that reduced visual phototransduction
324 or downstream neurotransmission cause sleep loss and/or fragmentation, indicating a clear role of
325 *Drosophila* photoreceptor R1-R6 in sleep promoting.

326 Our finding is consistent with previous publication that a visually impaired *omb* mutant showed
327 major sleep loss in both day and night (Kirszenblat et al., 2019) as well as the finding that activation
328 of L2 LMC neurons cause sleep lost (Muraro and Ceriani, 2015).

329 However our data is in contrary to the finding that blocking neural transmission for R1-R6 via TeTxLC
330 caused day sleep increase (Damulewicz et al., 2020). As mentioned above, we cannot produce viable
331 adult flies that express TeTxLC in photoreceptors and instead we apply Kir2 channel to hyperpolarise
332 photoreceptor. There may be two factors relate to these data discrepancy between us and
333 Damulewicz et al (2020): Firstly, our Gal4 and UAS lines are outcrossed to the w^{iso31C} background, as
334 a standard in the sleep research field, while such outcrossing were not described in Damulewicz et
335 al. Secondly, vision is important for navigation, and the compromised vision in phototransduction
336 mutants and flies with weaken photoreceptor outputs (i.e., *gmr-Gal4>UAS-kir2* or *gmr-Gal4>UAS-*
337 *TeTxLC*) can result in reduced wake locomotor activities, which is a major compounding factor in
338 assessing sleep increase. In fact, nine visual mutants in our survey ($ninaE^{5}$, $ninaE^{8}$, $norpA^{p24}$, trp^{1},
339 $trpl^{302}$, hdc^{JK910}, hdc^{MB}, ort^{attp} and *gmr-Gal4>uas-kir2*) showed significant reduced wake activity in
340 either day and/or night. Consequentially, the reduced wake activity prevents us to conclude whether
341 the increased night sleep seen in $ninaE^{8}$, $trpl^{302}$ and ort^{attp} are genuine increase or a reflection of loss
342 of locomotion during wakefulness. The wake activity was not analysed by Damulewicz et al (2020)

343 preventing a direct comparison between the two studies. More surprisingly, the sleep loss identified
344 in *hdc* and *ort* mutants is also opposite to previous finding indicating that histamine as pro-waking
345 factor (Oh et al., 2013). One major difference to Oh et al (2013) is that we only monitored male
346 sleeps in order to reach large range of sleep dynamics (see introduction). Importantly female flies
347 have very low levels of day sleep. Further investigation for female visual mutants will be required in
348 future to clarify if the role of visual input in sleep diverge between males and females. Nevertheless,
349 as shown in our data, three of the four mutants with histaminergic disruption show lower wake
350 activity, consistent with the arousal promoting role of histamine proposed by Oh et al (2013), yet
351 similar to Damulewicz et al (2020) Oh et al did not assess the impact of wake activity on sleep. Wake
352 activity is a classic but somehow ignored analytical tool in the *Drosophila* sleep research. We suggest
353 that this parameter should always be included for any locomotion-based sleep assay.

354 While uas-Kir2 has been used for previous sleep investigation, a *+>uas-Kir2* control were not
355 included (Shang et al., 2008). As mentioned, we observed an unexpected abnormal low night sleep
356 in *+>uas-Kir2* control flies which is likely due to leaking expression of Kir2 at other part of fly brain,
357 since the *gmr-Gal*4 and *UAS-Kir2* lines we used are isogenised to w^{iso31C} background. Nevertheless,
358 this limits our investigation to day sleep. Future investigation using gmr-LexA and LexAoP-Kir2 may
359 be required to verify this finding.

360 Unlike the isogenised Gal4 and UAS transgenic lines, the visual mutants come with various genetic
361 background including unknown *white* backgrounds and two wild type backgrounds. Due to the
362 complex mutagenic nature for these lines (point mutations or insertion) we were not able to bring all
363 these lines into common background. We therefore included in most cases at least two alleles and
364 most close background controls for sleep comparison, and we only interpreted the consistent
365 phenotype that presented across the alleles. Future investigation utilised the isogenised somatic
366 CRISPR knockout constructs will be required to rule out impact of background in this study.

367 As part of control experiments, we examined the ERG profiles for all the tested gene-specific
368 mutants. However, the severity of sleep loss phenotype does not directly correlate with ERG defects:
369 weakest $ninaE^{5}$ mutant (only on transient defected is detected) showed just as much day sleep
370 fragmentation as $ninaE^{8}$, $ninaE^{17}$ or $norpA^{p24}$. Moreover, trp^{1} has more dramatic defect in ERG as
371 compared to trp^{MB}; while trp^{1} showed reduce sleep in both day and night, trp^{MB} only showed night
372 sleep loss. Intriguingly both $trpl^{302}$ and $trpl^{MB}$ exhibit sleep loss, despite that our ERG recordings
373 showing minor or no impact of *trpl* mutants on ERG profile, in agreement with previous studies. As
374 mentioned above these divergent sleep phenotypes may be partly due to the various genetic
375 background we did not control as well as the expression profile outside photoreceptors. When

376 combining with Gal4-UAS investigation (Figure 5), it is nevertheless clear that R1-R6 photoreceptor is
377 required for driving day sleep. Vision and light-affected sleep are previously shown to be mediated
378 both via circadian clock neurons (eg., LNvs and DN1) as well as ring neurons in EB (Lamaze et al.,
379 2018; Raccuglia et al., 2019; Shang et al., 2008; Singh et al., 2023). Various neural pathways have
380 been shown to connect photoreceptors to the wake promoting LNv: R1-R6 photoreceptor can
381 propagate the light signal via histamine-mediated inhibition of the downstream LMC neurons that
382 activates LNvs (Muraro and Ceriani, 2015); on the other hand, R8 has recently been shown to
383 communicate with LNv via the so-called AMA neurons and cholinergic signalling. Moreover, sleep
384 promoting signal from photoreceptors may also reach EB via Anterior Visual Pathway through MeTu
385 and TuBu neurons, upon which circadian clock neuron DN1 also converge (Guo et al., 2018; Lamaze
386 et al., 2018). It is currently unclear which of the above pathways are required for the photoreceptor
387 mediated day sleep promotion. Although the day sleep loss observed in *ort*^{1}, *ninaE* mutants and *Rh1-*
388 *Gal4>uas-Kir2* indicates that the day sleep depends on Rh1-positive R1-R6 histaminergic inhibition
389 on LMC neurons, we did not observe consistent day sleep loss among *hdc* mutants. Nevertheless,
390 hdc expression is wider than visual system(Oh et al., 2013). Moreover, loss of hdc activity does not
391 completely remove histamine production (eg., Stenesen et al., 2015) and is not equal to selective
392 blockage of ort-mediated histamine synaptic transmission. Five tested mutants also exhibit night
393 sleep loss (two *hdc* mutants, *norpA*^{p24}, *trp*^{1} and *trp*^{MB}). One common feature of these five mutants is
394 that they may disrupt R7/R8 photoreceptors function in addition to R1-R6 photoreceptors.
395 Rhodopsin 3-expressing R7 was previously shown to selectively control night sleep (Damulewicz et
396 al., 2020). R8 was shown to synapse onto L1 LMC neurons and downstream motion sensing network
397 (Davis et al., 2020b). Consistently, a previous study suggest that a homeostatic night sleep drive can
398 be imposed by imbalanced activation of downstream targets in the LMC mediated motion sensing
399 network (Kirszenblat et al., 2019). Due to the low night sleep observed in *+>uas-Kir2* flies, we cannot
400 assess the effect of photoreceptor inhibition in night sleep. It is possible the night sleep loss
401 observed in the five visual mutants are due to similar imbalance output from photoreceptor subsets.
402 In summary, our data indicates a clear sleep promoting role by *Drosophila* photoreceptor. The
403 revelation of a potential neural network demarcation of day and night sleep require further
404 investigation beyond this report.

405

Acknowledgements

This study was funded by University of Leicester to M.A. and X.L. (F50/100 PhD studentships), N. S., S. H., T. O., and J. A (PGT and UG programme funding), an Erasmus + studentship to C. S.-G., a Royal Society University Fellowship to B. W., and a BBSRC New Investigator Scheme (BB/W014939/1) to K-F.C and Y-C. H.

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.

422 **Figure captions**

423 **Figure 1. sleep profile, parameters and ERG quantification for *ninaE* mutants. A.** Sleep traces of
424 average sleep minutes per 30 minutes over 12 hours light-dark cycle. ZT0: light on, ZT12: light off,
425 ZT24: end of night. Sleep traces of *iso* control and *ninaE* mutants are colour coded in the shades of
426 greys and reds. **B.** Average day sleep bout length (minutes), **C.** Average day wake activity (beam
427 breaks per waking minutes), **D.** Average day sleep (minutes), **E.** Average night sleep bout length
428 (minutes), **F.** Average night wake activity (beam breaks per waking minutes), and **G.** Average night
429 sleep (minutes) for $ninaE^{17}$ (i), $ninaE^{8}$ (ii) and $ninaE^{5}$ (iii) compared to iso controls. Values for each
430 individual are plotted as filled circles except for those outside (B-G). **H.** An example ERG profile from
431 iso control with 1000 ms green light flash that evokes voltage changes of on-transient, receptor
432 potential and off-transient. **I.** Mean and standard deviation for voltage change (mV) in on transient,
433 receptor potential and off-transient of ERG upon four light intensity (0.004, 0.02, 0.1 and 0.5 w/m^2)
434 flash for $ninaE^{17}$ and controls. **J.** Mean and standard deviation for voltage change (mV) in on
435 transient, receptor potential and off-transient of ERG upon four light intensity (0.004, 0.02, 0.1 and
436 0.5 w/m^2) 1000ms flash for $ninaE^{5}$, $ninaE^{8}$ and controls. Plot scales are in either liner (A-G) and
437 logarithmic (I-J). Asterisk indicates statistical significance ($p<0.05$) between mutants and iso controls
438 by pairwise Wilcoxon rank sum exact test without (B-H) and with multiple comparison, Benjamini-
439 Hochberg correction (I, followed by Kruskal-Wallis rank sum test). See **Table 1** for sample size, p-
440 values and effect size for each comparison.

441 **Figure 2. sleep profile, parameters and ERG quantification for *norpA* mutants. A.** Sleep traces of
442 average sleep minutes per 30 minutes over 12 hours light-dark cycle. ZT0: light on, ZT12: light off,
443 ZT24: end of night. Sleep traces for *iso* control and $norpA^{p24}$ mutants are colour coded in grey and
444 cyan. **B.** Average day sleep bout length (minutes), **C.** Average day wake activity (beam breaks per
445 waking minutes), **E.** Average night sleep bout length (minutes), **F.** Average night wake activity (beam
446 breaks per waking minutes), **G.** Average night sleep (minutes) for $norpA^{p24}$ compared to iso controls.
447 Values for each individual are plotted as filled circles except for those outside (B-G). **H.** mean and
448 standard deviation for voltage change (mV) in on transient, receptor potential and off-transient of
449 ERG upon four light intensity (0.004, 0.02, 0.1 and 0.5 w/m^2) flash for $norpA^{p24}$ and *iso* controls. Plot
450 scales are in either liner (A-G) and logarithmic (H). Asterisk indicates statistical significance ($p<0.05$)
451 between mutants and iso controls by pairwise Wilcoxon rank sum exact test. See **Table 2** for sample
452 size, p-values and effect size for each comparison.

453 **Figure 3. sleep profile, parameters and ERG quantification for *trp* and *trpl* mutants. A.** Sleep traces
454 of average sleep minutes per 30 minutes over 12 hours light-dark cycle. ZT0: light on, ZT12: light off,

455 ZT24: end of night. Sleep traces for *Canton S* (i), *Oregon R* (i) and *iso* controls (ii-iv) are coloured
456 coded in the shades of greys; *trp* (i-ii) and *trpl* (iii-iv) mutants are colour coded in shades of blue and
457 purples. **B.** Average day sleep bout length (minutes), **C.** Average day wake activity (beam breaks per
458 waking minutes), **D.** Average day sleep (minutes), **E.** Average night sleep bout length (minutes), **F.**
459 Average night wake activity (beam breaks per waking minutes), **G.** Average night sleep (minutes) for
460 trp^{1} (i), trp^{MB} (ii), $trpl^{302}$ (iii) and $trpl^{302/MB}$ or $trpl^{MB}$ (iv) compared to controls. Values for each
461 individual are plotted as filled circles except for those outside (B-G). **H.** Mean and standard deviation
462 for voltage change (mV) in on transient, receptor potential and off-transient of ERG upon four light
463 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)
464 compared to controls. Plot scales are in either liner (A-G) and logarithmic (H). Asterisk indicates
465 statistical significance ($p<0.05$) between mutants and controls by pairwise Wilcoxon rank sum exact
466 test without (Aii-Gii, Aiii-Giii, Hi and Hii) and with multiple comparison, Benjamini-Hochberg
467 correction (Ai-Gi, Aiv-Giv, and Hiii followed by Kruskal-Wallis rank sum test). See **Table 3 and Table 4**
468 for sample size, p-values and effect size for each comparison.

469 **Figure 4. sleep profile, parameters and ERG quantification for *hdc* and *ort* mutants. A.** Sleep traces
470 of average sleep minutes per 30 minutes over 12 hours light-dark cycle. ZT0: light on, ZT12: light off,
471 ZT24: end of night. Sleep traces for *Canton S* (i) and *iso* controls (ii-iii) are coloured coded in the
472 shades of greys; *hdc* (i) and *ort* (ii-iii) mutants are colour coded in shades of indigos and greens. **B.**
473 Average day sleep bout length (minutes), **C.** Average day wake activity (beam breaks per waking
474 minutes), **D.** Average day sleep (minutes), **E.** Average night sleep bout length (minutes), **F.** Average
475 night wake activity (beam breaks per waking minutes), **G.** Average night sleep (minutes) for *hdc*
476 mutants (i), ort^{1} (ii) and $ort^{attp/+}$ or ort^{attp} (iii) compared to controls. Values for each individual are
477 plotted as filled circles except for those outside (B-G). **H.** Mean and standard deviation for voltage
478 change (mV) in on transient, receptor potential and off-transient of ERG upon four light intensity
479 (0.004, 0.02, 0.1 and 0.5 w/m^2) flash for *hdc* (i) and *ort* (ii) mutants compared to controls. Plot scales
480 are in either liner (A-G) and logarithmic (H). Asterisk indicates statistical significance ($p<0.05$)
481 between mutants and controls by pairwise Wilcoxon rank sum exact test without (Aii-Gii) and with
482 multiple comparison, Benjamini-Hochberg correction (Ai-Hi, Aiii-Giii, and Hii followed by Kruskal-
483 Wallis rank sum test). See **Table 5** for sample size, p-values and effect size for each comparison.

484 **Figure 5. sleep profile and parameters for flies with hyperpolarised photoreceptors. A.** Sleep traces
485 of average sleep minutes per 30 minutes over 12 hours light-dark cycle. ZT0: light on, ZT12: light off,
486 ZT24: end of night. Sleep traces for *gmr-Gal4>+* (i), *Rh1-Gal4>+* (ii) and *+>UAS-Kir2* (i-ii) control flies
487 are coloured coded in the shades of greys; experimental mutants *gmr-Gal4> UAS-kir2* (i) and *Rh1-*
488 *Gal4>UAS-kir2* (ii) are colour coded in light burgundy. **B.** Average day sleep bout length (minutes), **C.**

489 Average day wake activity (beam breaks per waking minutes), **D.** Average day sleep (minutes), **E.**
490 Average night sleep bout length (minutes), **F.** Average night wake activity (beam breaks per waking
491 minutes), **G.** Average night sleep (minutes) for *gmr-Gal4> UAS-kir2* (i) and *Rh1-Gal4>UAS-kir2* (ii)
492 compared to controls. Values for each individual are plotted as filled circles except for those outside
493 (B-G). Asterisk indicates statistical significance ($p<0.05$) between mutants and controls by pairwise
494 Wilcoxon rank sum exact test with multiple comparison, Benjamini-Hochberg correction (followed
495 by Kruskal-Wallis rank sum test). See **Table 6** for sample size, p-values and effect size for each
496 comparison.

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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	24	12	large
on transient	*iso* vs *ninaE*^{17}	1.90E-08	0.78	24	12	large
off transient	*iso* vs *ninaE*^{17}	1.60E-09	0.81	24	12	large
receptor potential	*iso* vs *ninaE*^{5}	4.40E-01	0.17	12	12	
receptor potential	*iso* vs *ninaE*^{8}	4.40E-06	0.83	12	12	large
receptor potential	*ninaE*^{5} vs *ninaE*^{8}	4.40E-06	0.84	12	12	large
on transient	*iso* vs *ninaE*^{5}	1.70E-03	0.64	12	12	large
on transient	*iso* vs *ninaE*^{8}	4.40E-06	0.84	12	12	large
on transient	*ninaE*^{5} vs *ninaE*^{8}	5.97E-02	0.39	12	12	
off transient	*iso* vs *ninaE*^{5}	1.70E-02	0.48	12	12	moderate
off transient	*iso* vs *ninaE*^{8}	1.10E-06	0.85	12	12	large
off transient	*ninaE*^{5} vs *ninaE*^{8}	1.10E-06	0.85	12	12	large

760 **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	16	16	large
on transient	*iso* vs $norpA^{p24}$	3.30E-09	0.85	16	16	large
off transient	*iso* vs $norpA^{p24}$	1.50E-06	0.85	16	16	large

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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	12	12	large
on transient	*iso* vs trp^{1}	2.20E-05	0.78	12	12	large
off transient	*iso* vs trp^{1}	7.40E-07	0.85	12	12	large
receptor potential	*iso* vs trp^{MB}	4.40E-03	0.53	12	16	large
on transient	*iso* vs trp^{MB}	2.60E-07	0.83	12	16	large
off transient	*iso* vs trp^{MB}	5.70E-01	0.11	12	16	

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	24	12	
receptor potential	*iso* vs $trpl^{MB}$	8.60E-01	0.03	24	20	
receptor potential	$trpl^{302}$ vs $trpl^{MB}$	2.20E-01	0.26	12	20	
on transient	*iso* vs $trpl^{302}$	2.40E-06	0.73	24	12	large
on transient	*iso* vs $trpl^{MB}$	1.50E-01	0.22	24	20	
on transient	$trpl^{302}$ vs $trpl^{MB}$	1.00E-05	0.80	12	20	large
off transient	*iso* vs $trpl^{302}$	4.80E-09	0.81	24	12	large
off transient	*iso* vs $trpl^{MB}$	9.90E-01	0.00	24	20	
off transient	$trpl^{302}$ vs $trpl^{MB}$	5.30E-08	0.81	12	20	large

845 **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 hdc^{JK910}	9.50E-09	0.81	16	24	large
day sleep	hdc^{MB} vs *iso*	6.10E-05	0.67	16	23	large
day sleep	hdc^{JK910} vs *iso*	7.30E-05	0.60	24	23	large
night sleep	hdc^{MB} vs hdc^{JK910}	1.70E-04	0.61	16	24	large
night sleep	hdc^{MB} vs *iso*	5.40E-08	0.78	16	23	large
night sleep	hdc^{JK910} vs *iso*	4.99E-03	0.42	24	23	moderate
day sleep bout length	hdc^{MB} vs hdc^{JK910}	5.10E-07	0.76	16	24	large
day sleep bout length	hdc^{MB} vs *iso*	4.90E-03	0.49	16	23	moderate
day sleep bout length	hdc^{JK910} vs *iso*	1.08E-01	0.25	24	23	
night sleep bout length	hdc^{MB} vs hdc^{JK910}	8.50E-01	0.03	16	24	
night sleep bout length	hdc^{MB} vs *iso*	3.30E-05	0.64	16	23	large
night sleep bout length	hdc^{JK910} vs *iso*	2.50E-06	0.66	24	23	large
day wake activity	hdc^{MB} vs hdc^{JK910}	7.10E-01	0.06	16	24	
day wake activity	hdc^{MB} vs *iso*	1.40E-01	0.27	16	23	
day wake activity	hdc^{JK910} vs *iso*	2.50E-01	0.19	24	23	
night wake activity	hdc^{MB} vs hdc^{JK910}	2.58E-02	0.36	16	24	moderate
night wake activity	hdc^{MB} vs *iso*	1.02E-02	0.43	16	23	moderate
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	12	12	
receptor potential	*iso* vs hdc^{MB}	4.00E-01	0.06	12	12	
receptor potential	hdc^{JK910} vs hdc^{MB}	3.80E-01	0.32	12	12	
on transient	*iso* vs hdc^{JK910}	4.20E-05	0.72	12	12	large
on transient	*iso* vs hdc^{MB}	2.20E-04	0.79	12	12	large
on transient	hdc^{JK910} vs hdc^{MB}	9.32E-01	0.02	12	12	
off transient	*iso* vs hdc^{JK910}	5.50E-05	0.85	12	12	large
off transient	*iso* vs hdc^{MB}	2.20E-06	0.85	12	12	large
off transient	hdc^{JK910} vs hdc^{MB}	4.80E-04	0.72	12	12	large

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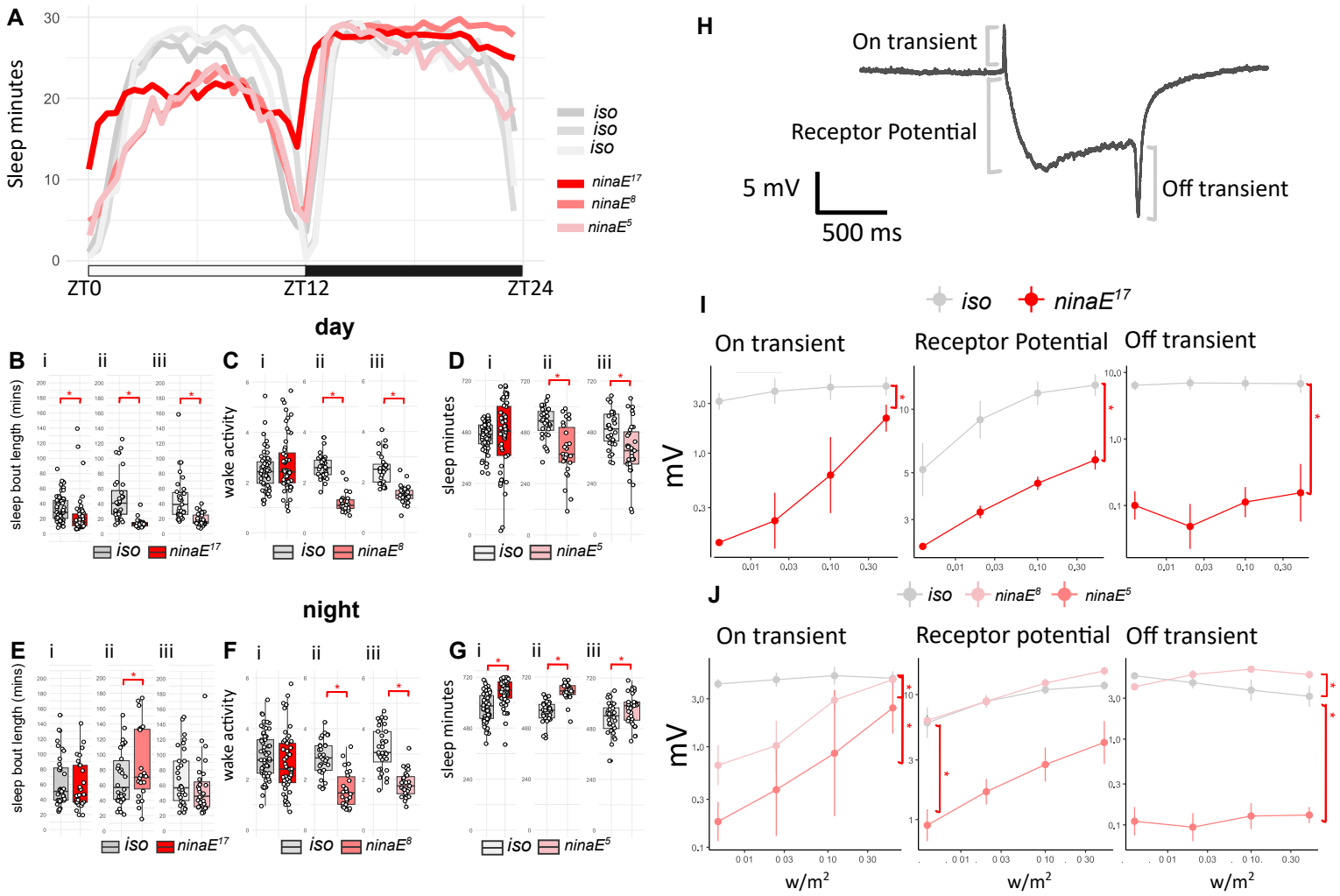
Table 5. Statistics of a. sleep and b. ERG parameters for *ort* mutants

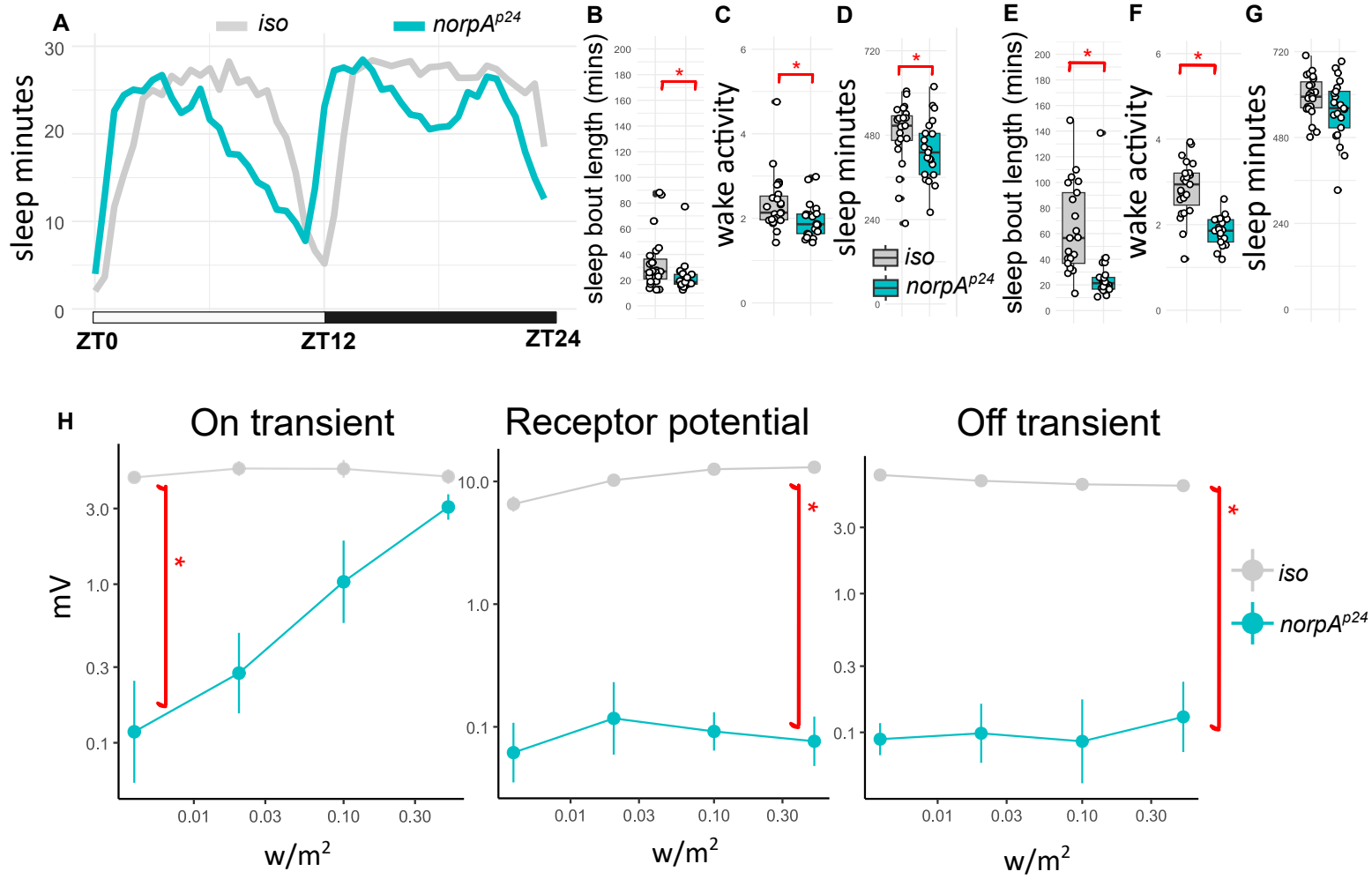
a. sleep parameter	genotype comparison	p-value	effect size *r*	n1	n2	significant *r* magnitude
day sleep	*Canton S* vs ort^{1}	5.20E-03	0.27	60	47	small
night sleep	*Canton S* vs ort^{1}	1.00E-01	0.16	60	47	
day sleep bout length	*Canton S* vs ort^{1}	2.60E-04	0.35	60	47	moderate
night sleep bout length	*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

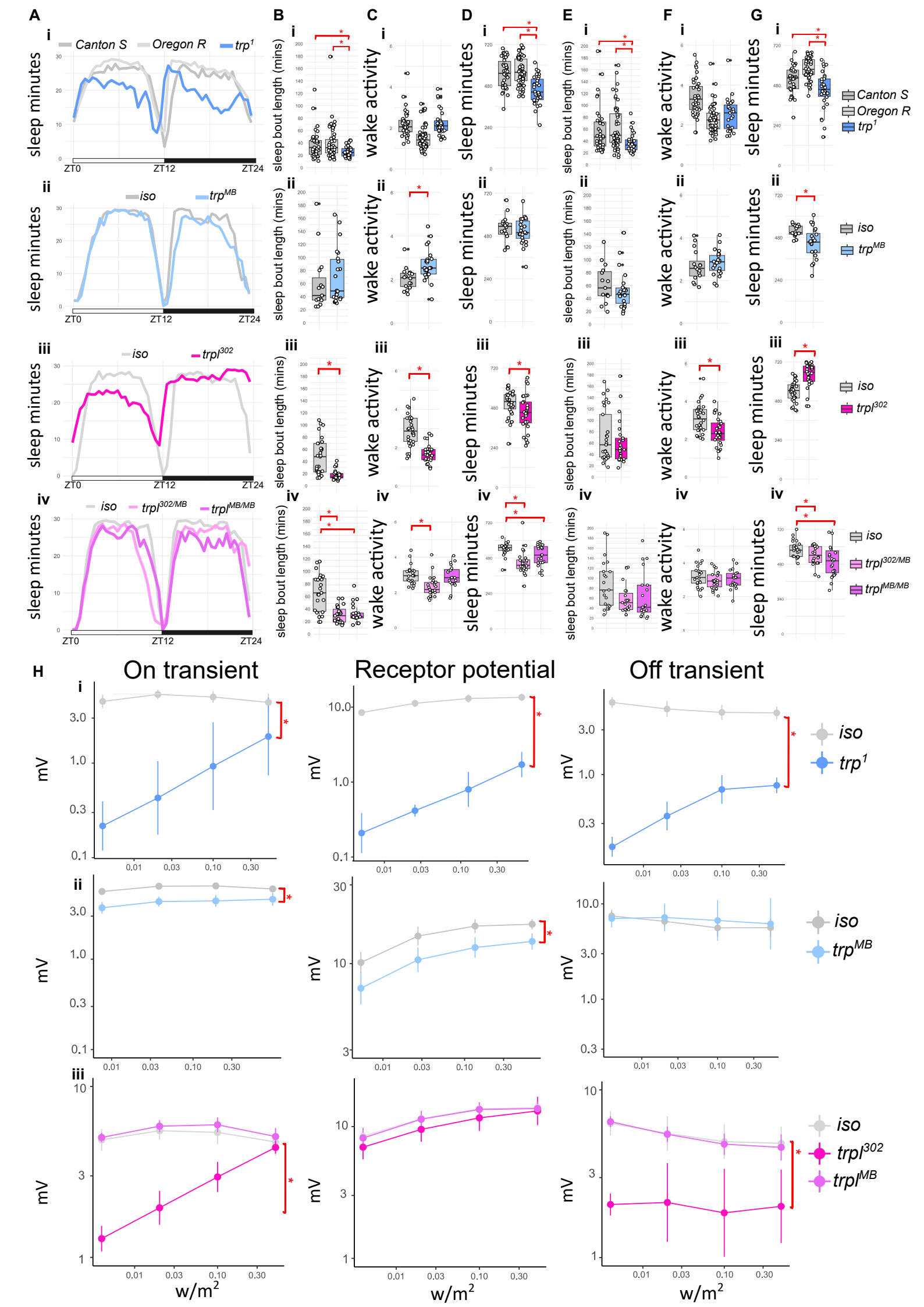
b.ERG parameter	genotype comparison	p-value	effect size *r*	n1	n2	significant *r* magnitude
receptor potential	*iso* vs ort^{1}	2.70E-08	0.83	20	12	large
receptor potential	*iso* vs ort^{attp}	9.80E-01	0.01	20	12	
receptor potential	ort^{1} vs ort^{attp}	1.10E-06	0.85	12	12	large
on transient	*iso* vs ort^{1}	9.30E-08	0.81	20	12	large
on transient	*iso* vs ort^{attp}	9.30E-08	0.81	20	12	large
on transient	ort^{1} vs ort^{attp}	2.20E-01	0.26	12	12	
off transient	*iso* vs ort^{1}	2.70E-08	0.83	20	12	large
off transient	*iso* vs ort^{attp}	4.90E-06	0.83	20	12	large
off transient	ort^{1} vs ort^{attp}	7.90E-01	0.06	12	12	

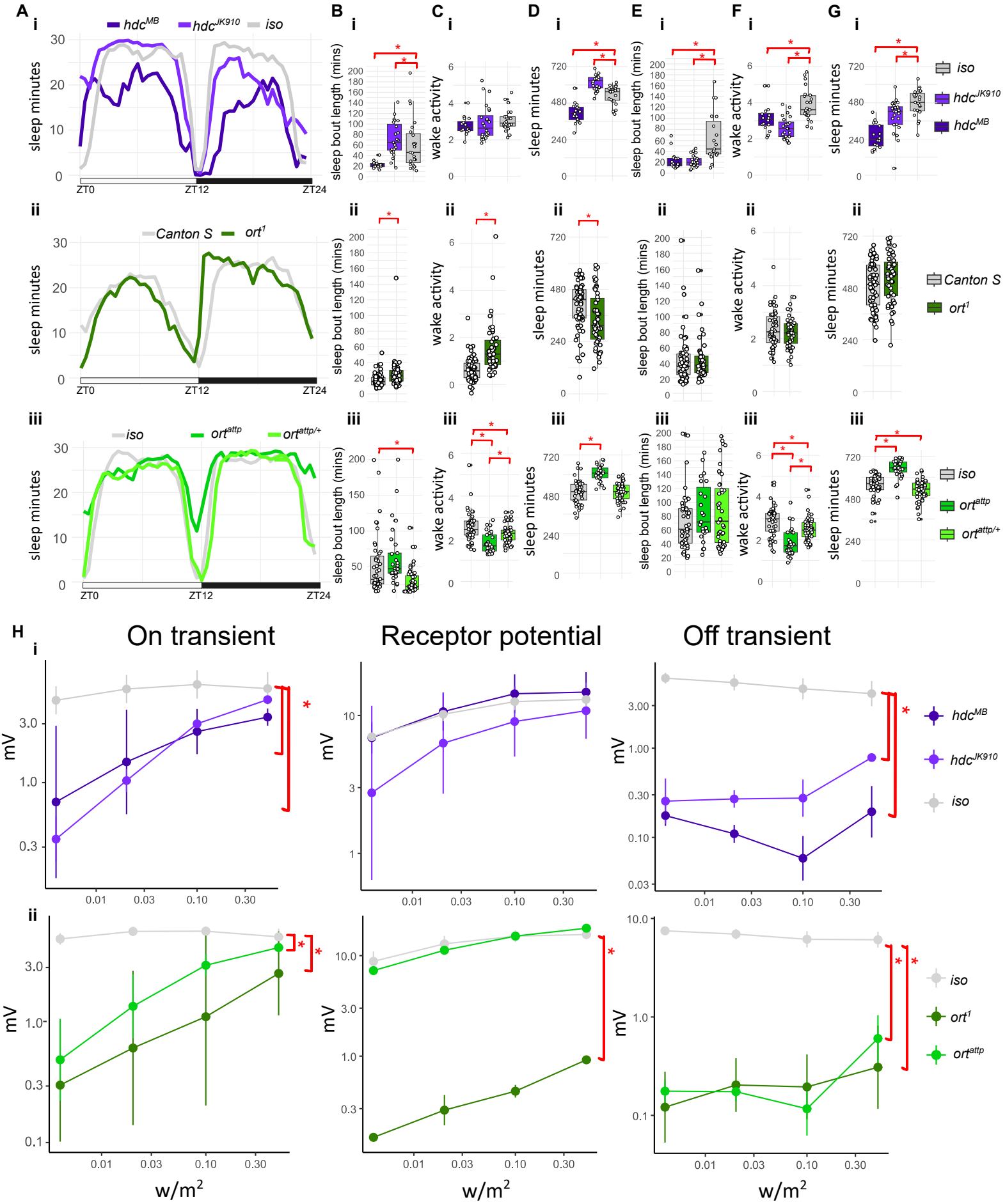
Table 6. Statistics of sleep parameters for *gmrGal4>UAS-Kir2 and Rh1Gal4>UAS-Kir2* lines

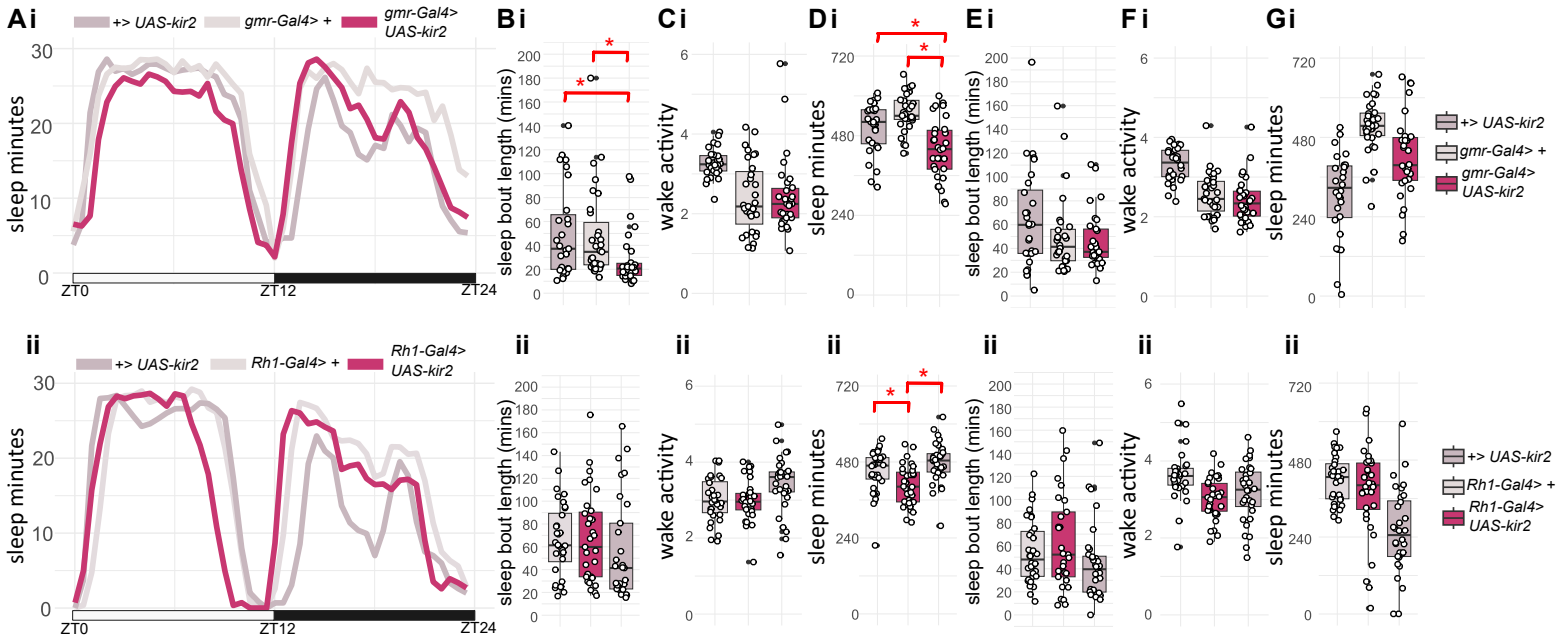
sleep parameter	genotype comparison	p-value	effect size *r*	n1	n2	significant *r* magnitude
day sleep	*gmr-Gal4>+* vs *gmr-Gal4>UAS-Kir2*	6.90E-03	0.36	32	32	moderate
day sleep	*gmr-Gal4>+* vs *+>UAS-Kir2*	1.18E-01	0.20	32	32	
day sleep	*gmr-Gal4>UAS-Kir2* vs *+>UAS-Kir2*	1.50E-04	0.51	32	32	large
night 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	


A
Sleep minutes
30
20
10
0
ZT0
ZT12
ZT24
iso
iso
iso
ninaE17
ninaE8
ninaE5
day
B
C
D
sleep bout length (mins)
wake activity
sleep minutes
iso
ninaE17
ninaE8
ninaE5
night
E
F
G
H
On transient
Receptor Potential
Off transient
5 mV
500 ms
I
iso
ninaE17
On transient
Receptor Potential
Off transient
mV
J
iso
ninaE8
ninaE5
On transient
Receptor potential
Off transient
mV
w/m2


A
iso
norpA^p24
sleep minutes
ZT0
ZT12
ZT24
B
sleep bout length (mins)
C
wake activity
D
sleep minutes
iso
norpA^p24
E
sleep bout length (mins)
F
wake activity
G
sleep minutes
H
On transient
Receptor potential
Off transient
mV
w/m2
iso
norpA^p24


A
B
C
D
E
F
G
H
sleep minutes
sleep bout length (mins)
wake activity
ZT0
ZT12
ZT24
Canton S
Oregon R
trp1
iso
trpMB
trpl302
trpl302/MB
trplMB/MB
On transient
Receptor potential
Off transient
mV
w/m2


A
B
C
D
E
F
G
H
i
ii
iii
hdc^MB
hdc^JK910
iso
Canton S
ort^1
ort^attp
ort^attp/+
sleep minutes
sleep bout length (mins)
wake activity
ZT0
ZT12
ZT24
On transient
Receptor potential
Off transient
mV
w/m^2


Ai
+> UAS-kir2
gmr-Gal4> +
gmr-Gal4> UAS-kir2
sleep minutes
ZT0
ZT12
ZT24
Bi
sleep bout length (mins)
Ci
wake activity
Di
sleep minutes
Ei
sleep bout length (mins)
Fi
wake 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
ii
sleep bout length (mins)
ii
wake activity
ii
sleep minutes
ii
sleep bout length (mins)
ii
wake activity
ii
sleep minutes
+> UAS-kir2
Rh1-Gal4> +
Rh1-Gal4> UAS-kir2