Sleep regulates visual selective attention in *Drosophila*

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Key words: attention, sleep, *Drosophila*, flies, behaviour

Summary statement: Sleep deprivation specifically impairs visual selective attention in fruit flies, without affecting behavioural responses to simple visual stimuli.

Abstract

Although sleep-deprivation is known to impair attention in humans and other mammals, the underlying reasons are not well understood, and whether similar effects are present in non-mammalian species is not known. We therefore sought to investigate whether sleep is important for optimising attention in an invertebrate species, the genetic model *Drosophila melanogaster*. We developed a high-throughput paradigm to measure visual attention in freely-walking *Drosophila*, using competing foreground/background visual stimuli. We found that whereas sleep-deprived flies could respond normally to either stimulus alone, they were more distracted by background cues in a visual competition task. Other stressful manipulations such as starvation, heat exposure, and mechanical stress had no effects on visual attention in this paradigm. In contrast to sleep-deprivation, providing additional sleep using the GABA-A agonist 4,5,6,7-tetrahydroisoxazolo-[5,4-c]pyridine-3-ol (THIP) did not affect attention in wild-type flies, but significantly improved attention in the learning mutant *dunce*. Our results reveal a key function of sleep in optimising attention processes in *Drosophila*, and establish a behavioural paradigm that can be used to explore the molecular mechanisms involved.

Introduction

The restorative effect of a night's sleep seems obvious, yet we still know very little about the function of sleep and how it impacts our behaviour. Studies in humans and other animals suggest that a fundamental function of sleep is to preserve cognitive functions such as learning, memory and attention (Stickgold 2005). This implies that sleep promotes brain plasticity, for example by strengthening neuronal circuits to consolidate memories (Diekelmann & Born 2010), or by maintaining optimal levels of neuronal functions by globally altering synaptic strengths (Tononi & Cirelli 2013). If a major function of sleep is to promote plasticity, a brain process that may be particularly vulnerable to sleep loss is selective attention. Given that selective attention requires precise temporal coordination between different neural populations (Fries et al. 2001; Elhilali et al. 2009), it may be most vulnerable to the changes in neural processing that may accrue when sleep homeostasis mechanisms are not in place (Kirszenblat & van Swinderen 2015). Consistent with this idea,

31 sleep-deprivation in humans leaves basic sensory processing intact (Casagrande et al. 2006;
32 Killgore 2010; Kendall et al. 2006), whereas tasks that involve high attentional load are
33 impaired (Chee & Chuah 2007; Kong et al. 2011). For this reason, sleep and attention may
34 share a deeper relationship than previously thought, and one that has not been thoroughly
35 investigated in genetic models such as *Drosophila*.

36 In *Drosophila*, sleep-deprivation has been associated with deficits in a variety of behaviours.
37 Sleep-deprivation impacts olfactory and visual memory in *Drosophila* (Glou et al. 2012;
38 Seugnet et al. 2008), as well as courtship memory (Ganguly-Fitzgerald et al. 2006) and
39 aggression in male flies (Kayser et al. 2015). In contrast to sleep-deprivation, induction of
40 sleep has been shown to reverse memory deficits in flies with genetic lesions and in a fly
41 model of Alzheimer's disease (Dissel, Melnattur, et al. 2015; Dissel, Angadi, et al. 2015). This
42 suggests that sleep can be used as a powerful therapeutic to enhance memory. As the
43 ability to pay attention is a prerequisite for all of the aforementioned behaviours, we
44 questioned whether optimising selective attention may be a key function of sleep in
45 *Drosophila*.

46 In this study, we developed a simple, high-throughput method to study visual selective
47 attention in flies. We found that whereas sleep-deprivation did not affect simple visual
48 behaviours (optomotor and fixation), sleep-deprived flies were more distractible in a visual
49 attention task involving competing stimuli. Normal attention was restored following sleep,
50 indicating that sleep promotes behavioural plasticity. In contrast to sleep-deprivation, we
51 found that inducing additional sleep using the GABA-A agonist 4,5,6,7-tetrahydroisoxazolo-
52 [5,4-c]pyridine-3-ol (THIP) had no effect on wild-type flies but could restore normal
53 attention to *dunce* learning mutants. Together, our results suggest that sleep optimises
54 selective attention processes in *Drosophila*.

55

56 **<u>Methods</u>**

57

58 **Fly stocks**

59 Strains used in this study were *Canton-S* (*CS*) wild-type flies, *dunce*^{1} mutants (outcrossed to
60 CS), *rutabaga*^{2080}, *pdf-gal4* flies (Bloomington Stock Centre, Indiana), and *UAS-Chrimson*

(P[20xUAS-IVS-CsChrimson.mVenus]attp18) flies (a gift from Vivek Jayaraman, Janelia Farm Research Campus, Virginia, U.S.A).

Sleep and visual behaviour

Our visual arena was adapted from Buridan's paradigm (Götz 1980). Flies had their wings clipped on CO_2, at least 2 days prior to the experiment. During the experiment, flies walked freely on a round platform, 86mm in diameter, surrounded by a water-filled moat to prevent escape. An individual fly was only tested once in each experiment, such that it was not influenced by previous visual stimuli. The temperature of the arena was 24-26 °C during experiments. Each experiment lasted 3 minutes, and the visual stimuli were presented on the horizontal or the vertical axes in alternation. Optomotor experiments were conducted with clockwise and anticlockwise gratings for 1.5 minutes each. A camera (Sony Hi Resolution Colour Video Camera CCD-IRIS SSC-374) placed above the arena was used to detect the fly's movement on the platform at 30 frames per second, and open-source tracking software was used to record the position of the fly (Colomb et al. 2012). Sleep was quantified using the DART system as previously described (Faville et al. 2015) using the 5 minute criterion for sleep (Shaw et al. 2000; Hendricks et al. 2000).

Visual stimuli

Each LED panel comprised 1024 individual LED units (32 rows by 32 columns) and was computer-controlled with LED Studio software (Shenzen Sinorad, Medical Electronics, Shenzen, China). The LEDs had a refresh rate of 200 Hz, ensuring there was no background flicker visible to the flies. All visual stimuli were created in Vision Egg software (Maimon et al. 2008), written in Python programming language (Ferguson et al. 2017). The walls of the arena consisted of 6 LED panels of green (520nm) and blue (468nm) LEDs that formed a hexagon surrounding the moat (29cm diameter, 16cm height), and onto which the visual stimuli were presented. Fixation stimuli were two dark stripes 180° apart, each 9° in width and 45° in height from the centre of the arena. The fixation stripes ranged from 38 - 55° in height and 4 – 14° in width depending on the fly's position in the arena. For visual competition experiments, 7 Hz flickering stripes (targets) were overlayed on top of a 3 Hz grating (speed 54° s^{-1}, luminance 402 Lux) in the background. For visual experiments in which stationary stripes were used as a distractor, the target stripes were flickered at 3 Hz, and the distractor stripes were non-flickering. For experiments in which the luminance

contrast of the grating was increased, the increments used were 0, 75, 146, 224, 402, 473, 649, 730 Lux.

Sleep-deprivation

3-5 day old female CS flies with clipped wings were sleep-deprived for 24 hours from 11am until 11am the next day, and then tested in the visual arena immediately afterwards. Mechanical sleep-deprivation was achieved using a sleep nullifying apparatus (SNAP) device, which has been shown to sleep deprive flies without triggering stress responses (Shaw et al. 2002). The device tilted back and forth, forcefully knocking and displacing flies every 20s (or 10s where indicated). Flies were contained in vials (20 females, 5 males) during the sleep-deprivation, because containing them individually in small tubes affected their performance in the attention assay. During the experiments, flies waiting to be tested were gently handled at least every 3 minutes so that they could not fall asleep prior to testing.

Optogenetics

Flies were grown on standard (agar/yeast) medium until 2-3 days prior to an experiment, after which they were transferred to standard medium supplemented with 0.5µM all-*trans*-retinal. Chrimson was activated using Red-Orange LEDS (Luxeon Rebel, 617nm, 700mA, Phillips LXM2-PH021-0070) as previously reported (Klapoetke et al. 2014). Flies were illuminated with >1000 Lux from 4 LED arrays.

Pharmacology

THIP was administered to flies in standard food at 0.1mg/ml concentration for 2 days, and removed 1 hour prior to measuring performance, as described previously (Dissel et al., 2015).

Data Analyses

Analyses of visual responses were performed using CeTran (3.4) software (Colomb et al, 2012), as well as custom-made scripts in R programming language. Stripe deviation was calculated as the smallest angle between the fly's trajectory and either of the vertical stripes (Ferguson et al. 2017; Colomb et al. 2012). For optomotor responses, the angular velocity (turning angle in ° s^{-1}) in the direction of the moving grating was calculated. All sleep and arousal metrics were obtained through the DART software. Statistical analyses were performed using Prism, R and MATLAB software. Lillifors tests were performed to confirm

122 the normality of the data, and *t* tests or one-way ANOVAs were used to detect significant
123 differences between groups.

124

125 **Results**

126 **A visual attention paradigm for freely walking flies.**

127 Selective attention allows us to focus on a single object or group of objects, while ignoring
128 less salient information (La Berge 1983; Eriksen & St James 1986). To examine attention-like
129 behaviour in freely-walking *Drosophila*, we designed a paradigm to measure behavioural
130 responses of flies to competing visual stimuli, involving 'targets' and 'distractors'. We took
131 advantage of two robust visual behaviours in *Drosophila*: fixation – a fly's tendency to orient
132 and walk towards a visually salient object, and optomotor behaviour, whereby a fly will turn
133 in the same direction as wide-field motion to stabilize its visual surroundings (Heisenberg et
134 al. 1984). Fixation has been previously measured using Buridan's paradigm, where flies with
135 clipped wings walk back and forth between two opposing vertical black stripes (Götz 1980;
136 Ferguson et al. 2017). We modified this paradigm to measure behavioural responses to
137 competing visual stimuli, using two opposing stripes as 'targets' in the foreground and
138 adding a wide-field motion stimulus as the 'distractor' in the background (Fig. 1A). A similar
139 configuration has been used for 'Fig./ground' discrimination in tethered flight experiments
140 (Heisenberg et al. 1984; Fox et al. 2014; Fenk et al. 2014; Aptekar et al. 2015; Aptekar & Frye
141 2013), but this complex stimulus has not been tested in walking flies.

142 We measured fixation on the targets by calculating the deviation angle between the fly's
143 heading direction and the target stripe, with smaller angles indicating greater attention to
144 the target (Fig. 1B) (Colomb et al. 2012). Response to the distractor, the motion stimulus,
145 was measured by the angular velocity in the direction of motion (the turning angle s^{-1} (y):
146 Fig. 1B). First, we investigated visual responses to both stimuli alone, across a range of
147 frequencies (Fig. S1A,B). Flies fixated best on targets flickering in the range of 3 – 7 Hz (Fig.
148 S1A), while optomotor responses were highest at 16 Hz (Supplementary Fig. 1B). For visual
149 competition experiments, we used a target frequency of 7 Hz, as this stimulus was very
150 salient to the flies and has previously been found to evoke strong fixation behaviour (Paulk
151 et al. 2015). The distractor was a lower contrast grating that spanned the entire arena and

152 moved “behind” the flickering targets. We used a grating frequency of 3 Hz (54 ° s^{-1}) at a
153 mid-luminance contrast, since we found responses under these conditions were robust and
154 consistent among flies, eliciting circular walking responses (Fig. 1E), and provided a low
155 enough level of distraction for the flies such that they could still continue to fixate on the
156 targets. Adding this low-contrast motion distractor significantly reduced flies’ fixation on
157 the target stripes: the straight paths evident when the targets were presented alone (Fig.
158 1C) were replaced by a combination of straight paths and circular paths, often angled in the
159 direction of motion (Fig. 1D). We modified the salience of the grating distractor by adjusting
160 the luminance contrast in linear increments (see methods) (Fig. 1F,G). As expected, when
161 the grating had a higher contrast (more salient), the distraction effect increased (Fig. 1F).
162 Motion responses to the distractor also increased (Fig. 1G) but appeared to plateau earlier
163 than target deviation in response to increasing the distractor salience. This suggests that
164 increased target deviation (Fig. 1F) may be a more sensitive measure of the distractor effect
165 than increased optomotor behaviour (Fig. 1G). In summary, our results show that attention-
166 like responses towards fixed targets can be effectively modulated (and titrated) by a motion
167 distractor stimulus in freely walking flies.

168

169 **Sleep-deprived flies are more distractible.**

170 Studies in humans and other animals have shown that sleep loss can impair a variety of
171 cognitive behaviours, such as learning, memory and attention (Alhola & Polo-kantola 2007;
172 Drummond et al. 2012; Lim & Dinges 2010). Similarly, studies in *Drosophila* have indicated a
173 role for sleep in visual and olfactory memory (Glou et al. 2012; Li et al. 2009; Seugnet et al.
174 2008; Seugnet et al. 2011). However, it is not known whether sleep modulates attention-like
175 behaviour in *Drosophila*. To investigate whether sleep-deprivation affects visual attention,
176 we sleep-deprived flies for 24 hours using the Sleep Nullifying Apparatus (SNAP) according
177 to previous methods (Shaw et al. 2002). We first confirmed that flies were sleep-deprived
178 by examining their sleep rebound (*i.e.* an increase in sleep need following sleep-
179 deprivation). Following sleep-deprivation, sleep was monitored using the DART system
180 (Faville et al. 2015) across three consecutive days, while another group of flies had their
181 attention tested (Fig. 2A). As expected, sleep-deprived flies slept more than control flies the
182 day after sleep-deprivation (day 1) particularly during the daytime, whereas by days 2 and 3

183 there was no difference in sleep duration between the sleep-deprived and control flies (Fig.
184 2B).

185 Following sleep-deprivation we measured fixation to stationary targets and optomotor
186 responses to a moving grating, in scenarios in which the stimuli were presented alone or in
187 competition (with the grating at three different levels of luminance contrast of the
188 distractor) (Fig. 2D,E). Optomotor responses to the moving grating in all visual scenarios
189 were largely unaffected by sleep-deprivation (Fig. 2E), as were fixation responses in the
190 absence of the grating (Fig. 2D, first point from the left). Interestingly, the effect of sleep-
191 deprivation on fixation behaviour was only evident when the two visual stimuli were
192 combined (Fig. 2C), as indicated by significantly increased target deviation for all three
193 combined conditions (Fig. 2D). This suggests that sleep-deprived flies are specifically
194 affected in their response to visual competition. Finally, we tested whether flies could
195 recover from sleep-deprivation by allowing them to rest for 24 hours. For this experiment
196 (and all subsequent experiments involving visual competition), we used a grating of mid-
197 luminance contrast (indicated by the red arrow heads in Fig. 2E,F). Again, we found that
198 sleep-deprived flies were more distractible, but their attention returned to normal following
199 24 hours rest (Fig. 2F). Overall, our results suggest that sleep-deprivation alters visual
200 attention, and that this effect is reversible.

201 Although we saw no alterations to optomotor responses of sleep-deprived flies when the
202 grating was presented alone, we considered the possibility that the sleep-deprivation
203 phenotype may be caused by an increased sensitivity to motion, which could potentially
204 arise from the preceding 24hours of constant motion they experience in the SNAP
205 apparatus. However, flies sleep-deprived in 24hours of darkness showed a similar
206 impairment of attention compared to controls, suggesting that visual experience did not
207 play a role in the sleep-deprivation phenotype (Fig. S2). We next asked whether the
208 increased distractibility of sleep-deprived flies was specific to the motion stimulus, or
209 whether it could apply to other types of visual distractors. We used a stationary stripe as a
210 distractor that was identical to the target but less salient as it was non-flickering (Fig. 3A).
211 As with the moving grating in the preceding experiments, attention to the target could be
212 titrated by modulating the salience (*i.e.,* contrast) of the distractor (Fig. 3B,C). Interestingly,
213 increasing the salience of the distractor correspondingly increased distractibility (Fig. 3C).

214 Furthermore, consistent with our previous attention experiments, we found that sleep-
215 deprived flies similarly paid less attention to the target in the presence of this alternative
216 distractor object (Fig. 3D). Our observation that sleep-deprivation is similarly disrupted by
217 different types of distractors suggests that a common attention mechanism is affected,
218 which is not dependent on lower level visual responses.

219 Given that sleep-deprivation reduces overall activity, we wondered whether this may
220 explain the reduced orientation to salient features in the visual attention assay. To
221 investigate this, we performed a correlation analysis on all data, to see if walking speed of
222 individual flies may predict selective attention behaviour (target deviation). However, we
223 found no correlation between attention and walking speed for either sleep-deprived or
224 control groups (Fig. 4A,B).
225
226 Considering that the sleep-deprivation protocol might be stressful (it involves waking the
227 flies every 20 seconds by mechanical stimulation), we further examined the effects of
228 different types of stress on the flies' visual attention. This allowed us to answer two
229 questions: 1) whether attention phenotypes could be modulated by environmental stressors
230 (e.g. heat, mechanical stress and starvation), which would indicate that the sleep-
231 deprivation effect on attention may simply be caused by stressful situations, and 2) whether
232 the sleep-deprivation phenotype occurs under different environmental conditions *(i.e.,* how
233 robust the effect is). For all these experiments we measured flies' visual responses in the
234 combined stimulus paradigm (in which flies fixate on targets in the presence of distractors)
235 (Fig. 1A).

236 First, we measured the effect of heating flies to 31°C for 24 hours (Fig. 5A). Prolonged
237 exposure to heat had no effect on attention, whereas the increase in distractibility caused
238 by sleep-deprivation was still present under conditions of heat stress (Fig. 5A). This
239 indicates that heat does not influence attention in this paradigm, or the sleep-deprived
240 effects on attention. Next, we measured the effects of mechanical stress using a stimulation
241 protocol that would provide the same amount of stimulation experienced during the sleep-
242 deprivation protocol, but including rest periods for sleep. This was achieved by subjecting
243 flies to mechanical stimulation for one hour followed by rest for one hour, repeated across
244 24 hours, but with double the rate of stimulation (to keep the actual number of stimuli the

245 same as for the sleep-deprivation regime) (Fig. 5B). Unlike sleep-deprived flies, flies
246 subjected to this stimulation regime containing rest periods had normal attention, indicating
247 that the sleep-deprivation effect was not due to mechanical stress of perturbing the flies
248 (Fig. 5B). We next assessed the effects of starvation, as sleep is known to be affected by
249 food availability (Siegel et al, 2009). In *Drosophila*, food deprivation has been found to
250 suppress sleep (Keene et al. 2010). Feeding was assessed during the sleep-deprivation by
251 measuring the intake of food containing blue food dye and, as previously reported, sleep-
252 deprived flies showed similar food intake to controls (Thimgan et al. 2010). We then tested
253 whether depriving flies of food, with or without sleep-deprivation, affected visual attention.
254 The visual attention scores of starved controls were not different from those of fed controls,
255 suggesting that starvation *per se* does not impair visual attention (Fig. 5C). As expected,
256 sleep-deprivation impaired visual attention in both starved and fed conditions but,
257 interestingly, starvation was able to partially suppress this effect (Fig. 5C, "SD fed" vs "SD
258 starved"), suggesting that starvation may protect against the detrimental effects of sleep-
259 deprivation. In summary, our results show that sleep-deprivation consistently affects visual
260 attention, even under conditions of heat and starvation, but that visual attention is resistant
261 to the effects of a variety of stresses in the absence of sleep-deprivation. Therefore, sleep is
262 more important than lack of stress for maintaining optimal levels of visual attention.

263 Having found that sleep-deprivation affects visual attention, we also sought alternative
264 methods to sleep deprive flies, to confirm that the sleep-deprivation effect on attention was
265 not specific to our mechanical stimulation protocol. Pigment Dispersing Factor (PDF)
266 neurons in the optic lobes of the fly brain are known to promote wake (Parisky et al. 2008).
267 We hypothesised that activating these neurons for 24 hours would have wake-promoting
268 effects that may also impair visual attention. To test this, we transiently expressed a red-
269 light activated channelrhodopsin, Chrimson (Klapoetke et al. 2014), in the Pigment
270 Dispersing Factor (PDF) neurons, such that exposing flies to red light could trigger
271 wakefulness. We noticed that red light exposure caused a general decrease in sleep among
272 all flies (Fig. 6A). However, sleep loss was greater in flies that expressed the
273 channelrhodopsin (*pdf-gal4/+>uas-chrimson/+*) and were exposed to red light for 24 hours,
274 as they lost more than 55% of their sleep compared to non-exposed controls. When we
275 subsequently tested these flies for visual attention, we found that this method of sleep-

276 deprivation resulted in an increase in target deviation specifically in the flies that were kept
277 awake genetically, *pdf-gal4/+>uas-chrimson/+* (Fig. 6B,C). This provides further support for
278 the idea that sleep-deprivation increases distractibility, and is not specific to the method of
279 sleep-deprivation.

280

281 **Additional sleep improves attention in *dunce* mutants, but not wild-type flies.**

282 Considering that sleep-deprivation made flies more distractible, we next asked whether
283 *increasing* sleep had the opposite effect on visual attention, making flies *less* distractible.
284 The GABA-A agonist (THIP) has recently been used to induce sleep in *Drosophila* (Dissel,
285 Angadi, et al. 2015; Yap et al. 2017). This increase in sleep was shown to reverse memory
286 deficits in *Drosophila* learning mutants *dunce* and *rutabaga* (Dissel, Angadi, et al. 2015) and
287 in a *Drosophila* model of Alzheimer's disease (Dissel et al. 2017). As *dunce* and *rutabaga*
288 mutants have previously been found to have defective attention processes (van Swinderen
289 & Brembs 2010; van Swinderen 2007), we wondered whether their attention deficits could
290 also be rescued by induced sleep.

291 We first quantified sleep in *dunce* and *rutabaga* mutants, and found that both these
292 mutants slept significantly less than wild-type flies, with *dunce* flies sleeping less during the
293 day and the night, and *rutabaga* mutants sleeping less during the day (Fig. 7A,B). THIP could
294 then be effectively used to increase sleep in both wild-type and mutant flies, to a similar
295 level (Fig. 7C,D). We next tested whether inducing sleep affected the visual attention of
296 wild-type flies in our free-walking paradigm. THIP was fed to wild-type (CS) flies for two
297 days and removed one hour prior to testing their behaviour (the same procedure used by
298 (Dissel, Melnattur, et al. 2015). We performed a within-group experiment (the same flies
299 tested before and after induced sleep), and a between-group experiment (aged-matched
300 flies, with or without induced sleep). In wild-type flies we observed that distractibility
301 (deviation away from the targets) decreased slightly for those flies that that had been
302 induced to sleep more, in both experiments; however, this effect was not significant (Fig. 7E,
303 p=0.084 and p=0.187 for within group and between group comparisons, by t-test.).

304 We next tested whether increasing sleep altered attention in *dunce* and *rutabaga* mutants.
305 Under normal conditions (without increasing sleep), *dunce* and *rutabaga* flies were

306 significantly less attentive towards the visual targets compared to wild-type flies as
307 measured by increased target deviation (Fig. 7F, black bar (WT) compared to light blue bar
308 (*dnc* (-)) and yellow bar (*rut* (-))). Interestingly, THIP administration was able to significantly
309 reduce target deviation in *dunce* mutants but not *rutabaga* mutants (Fig. 7F), such that
310 attention in *dunce* mutants with induced sleep was not different from that of wild-type flies
311 (Fig. 7F, dark blue bar (*dnc* (+)) compared to black bar (WT(-)). This suggests that increasing
312 sleep may improve attention in some learning mutants but not others.

313

314 **Discussion**

315 Selective attention is crucial for discriminating important from irrelevant stimuli in our
316 environment, even for insects such as *Drosophila*. The selection and suppression dynamics
317 required for attention seem to have emerged in brains at the same time at which sleep
318 became important for maintaining attention-related behaviours (Kirszenblat & van
319 Swinderen 2015). In other words, some sleep functions may have evolved to optimise
320 attention. Here, we have developed a free-walking attention paradigm which allowed us to
321 obtain a functional readout of the effects of sleep-deprivation and sleep induction on visual
322 attention in *Drosophila*.

323

324 Selective attention must require a certain level of brain coordination to deal with competing
325 stimuli. This is because different stimuli may be processed by different brain regions; in our
326 attention paradigm, the optomotor and fixation responses that compete with each other
327 have been found to be driven to some degree by independent visual circuits (Bahl et al.
328 2013; Fenk et al. 2014). Furthermore, attention-like behaviour in the fly is associated with
329 increased coherence between brain regions (Paulk et al. 2015), suggesting greater synaptic
330 coordination. If sleep is required to maintain synaptic coordination across the brain, it
331 follows that tasks involving greater cognitive load may be more vulnerable to sleep loss.
332 Indeed, we found that although sleep-deprived flies showed normal responses to simple
333 visual stimuli, they had altered responses to visual competition.

334

335 One might still ask, whether sleep has a privileged role in regulating selective attention
336 relative to other high order cognitive functions. Sleep loss is known to affect a variety of

complex cognitive behaviours in humans, such as learning and memory, creative thinking, and even the ability to speak clearly or to appreciate humour (Harrison & Horne 1997; Killgore 2010; Kendall et al. 2006). Attention is probably integral to these complex processes because it allows us to filter out irrelevant information and to select the right actions. In light of this, we would suggest that selective attention is a key *mechanism* that is affected by sleep loss, which disrupts the brain's ability to prioritize competing information.

The sleep-deprivation effects we observed on attention seemed to be remarkably robust under different environmental conditions, as flies were still more distractible following sleep-deprivation in conditions of heat stress, darkness and starvation. Interestingly, although starvation and heat alone can promote waking in flies (Ishimoto et al. 2012; Keene et al. 2010), they did not appear to affect our attention readout, unlike the mechanical perturbation method of sleep-deprivation which is in standard use for *Drosophila*. It is possible that because starvation and heat are environmental stimuli that a fly encounters in natural situations, it has already evolved physiological protective mechanisms to cope with these kinds of stresses. This is supported by reports that heat stress response factors can protect against sleep-deprivation-induced lethality (Shaw et al., 2002), and that starvation disrupts sleep homeostasis (Keene et al. 2010; Thimgan et al. 2010). *Foraging* mutants of *Drosophila*, which tend to explore further for food, also appear to have reduced sleep need and are resistant to memory impairments caused by sleep loss (Donlea et al. 2012). More recently, it was also discovered that sexual arousal in male flies can suppress the need for sleep (Beckwith et al. 2017), and vice versa, sleep can inhibit male sexual behaviour (Chen et al. 2017). Overall, these studies suggest that sleep need is flexible and may compete with other survival needs such as food, sexual reproduction and the need to escape unfavourable environments (e.g. high temperatures). In future it would be interesting to further investigate how a fly's environment influences its attention.

Interestingly, *increasing* sleep was able to improve the attention of *dunce* mutants, but not wild-type flies or *rutabag*a mutants. The finding that wild-type flies did not show improved attention following induced sleep is consistent with previous reports that increasing sleep does not improve learning and memory in wild-type flies (Dissel, Angadi, et al. 2015). One interpretation is that attention is already optimal in wild-type *Drosophila*. In contrast, both

369 *rutabaga* and *dunce* mutants have previously been identified as having attention deficits
370 (van Swinderen 2007), and our result confirmed this finding in a free-walking attention
371 paradigm. However, it is not clear why inducing sleep would improve attention specifically
372 in *dunce* mutants, and not in *rutabaga* mutants. It is possible that this may relate to their
373 different sleep phenotypes – *dunce* mutants appear more severely sleep-deficient in our
374 DART system (including at night, which *rutabaga* mutants are not) meaning that THIP had a
375 greater ability to restore sleep to *dunce* mutants. Related to this, it is possible that the
376 attention deficits of these mutants are due to different underlying causes. For example, the
377 mutants may have poor attention because they sleep less, or they may sleep less because
378 they have poor attention.
379
380 How inducing sleep could improve attention, or other aspects of cognition such as learning
381 and memory, also remains unclear. There is some evidence that the sleep-promoting agent
382 THIP can interact with specific GABA-receptors to promote sleep in flies, via known sleep-
383 promoting neurons of the dorsal fan-shaped body (Dissel, Angadi, et al. 2015), but can also
384 modulate dopaminergic pathways to facilitate memories (Berry et al. 2015). Interestingly, a
385 recent study found that increasing sleep promoted survival of wild-type flies exposed to
386 oxidative stress (Hill et al. 2018). Whether increasing sleep in flies improves brain function
387 through specific circuits, or by general cellular mechanisms like reducing reactive oxygen
388 species (ROS) needs to be further investigated. Our study opens up an opportunity to
389 understand the molecules, circuits and environmental factors involved in the role of sleep in
390 optimising attention.
391

392 **Acknowledgements**

393 We would like to thank Rowan Tweedale for comments on the manuscript. We would like
394 to thank John John for help with behavioural experiments, and Richard Faville and Ben
395 Kottler for help with DART software. This work was supported by an NIH grant RO1
396 NS076980-01 to PJS and BVS and an Australian Research Council grant DP140103184 to BVS.

397 **Competing Interests**

398 The authors declare no competing interests.

399 **References**

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533

534

535 **Figure Legends:**

536

537 **Figure 1. A visual attention paradigm.** A) Diagram of set up. Flies walk on a platform
538 surrounded by a moat of water, with six LED panels forming walls onto which visual cues are
539 presented. Visual attention is measured by fixation on either of two opposing stripes
540 ('fixation targets') in the presence of a motion stimulus ('distractor'). B) Calculating fixation
541 and optomotor responses (adapted from Colomb et al, 2013). Target fixation is measured
542 by the stripe deviation (β), the angle between the fly's heading direction (e.g. red line from
543 A to B) and the centre of the target stripe that is in the direction of movement (dotted black
544 line). Response to the motion stimulus (optomotor) is determined by the fly's angular
545 velocity, i.e. the fly's turning angle (g)/second. C-E) Example traces of a fly's walking path in
546 response to flickering target stripes (C), a motion stimulus (D) or competing stimuli (E). F,G)
547 Quantification of target deviation (F) or optomotor response (G) in wild type Canton-S flies
548 when different visual cues were presented: fixation targets alone ('no grating') or fixation
549 targets combined with gratings of increasing salience (luminance contrast), or grating alone.
550 n>10 flies for each condition, *p<0.05,**p,0.01,***p<0.001 by t-tests between adjacent
551 conditions.

552

553 **Figure 2. Sleep deprivation makes flies more distractible.** A) Sleep deprivation protocol.
554 Flies were sleep deprived for 24 hours and visual attention was tested the following day
555 (day 1), and compared to control (non-sleep deprived flies). B) Flies subjected to the sleep
556 deprivation protocol in (A) showed a sleep rebound (increase in sleep) measured by the
557 difference in sleep compared to control (non-sleep deprived) flies. C) Example traces of
558 walking behaviour in a control and sleep deprived fly responding to competing visual cues.
559 D,E) Quantification of target deviation (D) or optomotor response (E) in control vs sleep
560 deprived flies responding to target objects alone (target only), competing visual cues
561 (objects and grating together, with three levels of grating salience), and grating alone
562 (motion only). F) Attention returns to normal levels in flies rested for 24hrs following sleep
563 deprivation (tested with objects and mid-salience grating). n= 60 flies in (A,B) n>16 flies for
564 each condition in (D,E) and n>12 flies in (F). Red arrowheads indicate the grating contrast
565 that was used for all subsequent experiments *p<0.05 (one-way Anova in F, t-test in E) and
566 **p <0.01, ***p <0.001 by t test between control and sleep deprived. Error bars show the
567 s.e.m.

568

569 **Figure 3. Sleep deprivation increases distractibility to stationary objects.** A) Diagram of
570 visual stimuli, with flickering target stripes and non-flickering distractor stripes. B) Example
571 traces of a flies responding to targets only, or targets with distractor stripes. C) Target
572 deviation scores with increasing distractor salience (luminance contrast to background). D)
573 Target deviation for controls and sleep deprived flies in the presence of distractor objects.
574 n<20 flies in (C) and 50 flies in (D). **p<0.001 by t-tests between adjacent groups in (C) and
575 sleep deprived and controls in (D).

576

577 **Figure 4. Target deviation in the attention paradigm is not correlated with walking speed**
578 **or sleep following sleep deprivation**. A,B) Correlation of walking speed and target deviation
579 in control (A) and sleep deprived (B) flies. n = 48 flies. Pearson's correlation coefficient ('R')
580 were not significant (shown on graphs, alongside p values), indicating no correlations were
581 found.

582 **Figure 5. Starvation, heat, and mechanical stress effects on visual attention.** Visual
583 attention was measured by orientation towards 7hz flickering object ('targets') with a mid-
584 luminance contrast grating distractor (see methods). A) Target deviation in flies that
585 underwent heat stress (31 degrees) for 24 hours with or without sleep deprivation. B)
586 Target deviation following 24hrs mechanical stimulation. C) Target deviation in flies that
587 were starved and/or sleep deprived for 24hrs. "Control fed" vs "control starved" groups
588 were not significantly different. n>17 flies in (A), n>28 flies in (B) n = 20 flies in (C).
589 **p<0.01, ***p<0.001 by One-way ANOVA. Error bars indicate the s.e.m.

590 **Figure 6. Activation of wake promoting circuits for 24 hours increases distractibility**. A)
591 Change in sleep caused by red-light exposure. n= 17 B) Change in attention (target
592 deviation) following 24 hrs red light exposure. n = 10. C) Representative traces of a control
593 fly (CS) compared to a sleep deprived fly (pdf-gal4>uas-chrimson) following red light
594 exposure. t tests compared red-light activated to non-activated controls. **p=0.002,
595 ***p<0.001, one-way ANOVA. Error bars show the s.e.m.

596 **Figure 7. Increasing sleep improved attention in *dunce* mutants.** A) Hourly sleep profiles
597 averaged across 24 hours for wild-type (Canton-S), dunce and rutabaga flies (n>50 flies). B)
598 Quantification of sleep duration averaged across the day and the night, from data in (A). C)
599 Sleep across 24 hours in wild-type flies, dunce and rutabaga mutants treated with the sleep
600 promoting agent, THIP. Wild-type sleep without THIP is shown for comparison (black trace).
601 n>20 flies. D) Quantification of day-time and night-time sleep in wild-type, dunce and
602 rutabaga flies with and without THIP. n>20 flies. E) Target deviation of wild-type (Canton-S)
603 flies on control (vehicle) or THIP (0.1mg/ml) food. THIP was administered for 2 days and flies
604 were removed from THIP 1 hr prior to the visual attention test. 'Within group' compared
605 flies before and after THIP administration (n=11 flies), and 'between group' compared age-
606 matched flies fed control (vehicle) or THIP food (n = 8 flies). F) Stripe deviation in dunce
607 mutants before and after 2 days of THIP (n>30 for dunce and CS, n> 16 for rutabaga).
608 *p<0.05, ***p<0.0001 by one-way ANOVA (A-D) and t-test (E-F). Error bars show the s.e.m.

609

610 **Figure S1. Visual responses are modulated by flicker and grating frequency**. (A) Visual
611 fixation in wild-type flies responding to fixation targets that were static (0 Hz) or flickering at
612 0.5 Hz, 3 Hz, 7 Hz, or 16 Hz. Lower target deviation indicates greater fixation. n = 14 flies for
613 each condition. Asterisks indicate significance between adjacent data points (2-way ANOVA
614 with Tukey's correction). (B) Optomotor response in wild-type flies responding to gratings
615 that were stationary (0 Hz) or rotating at 0.5 Hz, 3 Hz, 7 Hz, or 16 Hz. Higher optomotor
616 index (OI) indicates greater optomotor response. n = 10. Asterisks indicate significance
617 between adjacent points (A), significance via 2-way ANOVA with Tukey's correction. * p
618 < .05; ** p< .01; *** p< .001. Error bars show S.E.M.

619 **Figure S2. Sleep deprivation effects on attention are independent of visual experience.**
620 Target deviation during figure/ground discrimination in flies that were sleep deprived for 24
621 hrs in normal circadian conditions (light) or in 24 hrs of darkness (dark). The visual stimuli
622 were a 7 Hz flickering object ('target') and a mid luminance contrast grating (see methods).
623 n>19 flies per data set. *p=0.03,**p=0.01 by t-test. Error bars indicate the s.e.m.

Figure 1

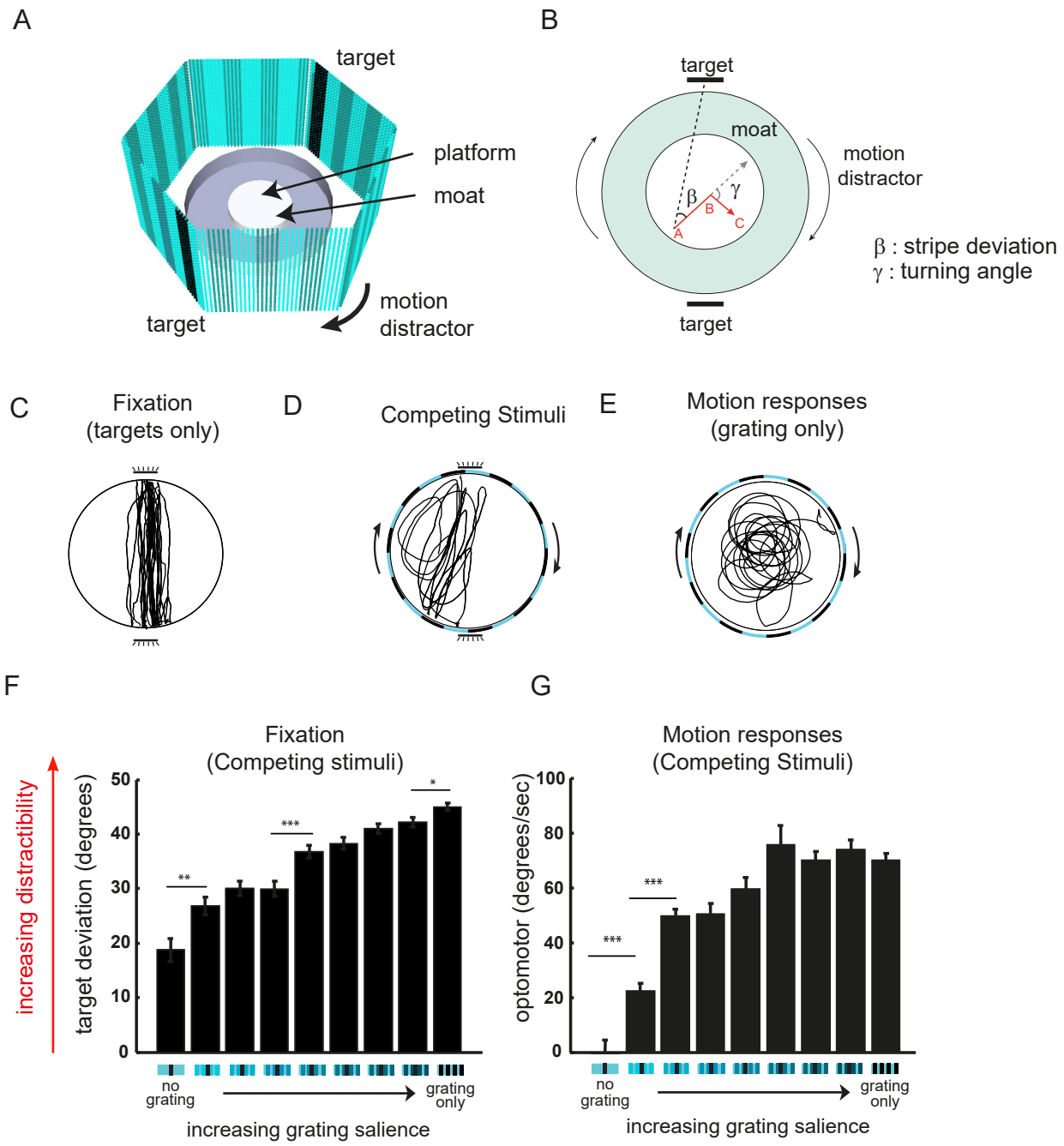

A
target
platform
moat
target
motion
distractor
B
target
moat
motion
distractor
β
γ
A
B
C
β : stripe deviation
γ : turning angle
target
C
Fixation
(targets only)
D
Competing Stimuli
E
Motion responses
(grating only)
F
Fixation
(Competing stimuli)
increasing distractibility
target deviation (degrees)
50
40
30
20
10
0
**
***
*
no
grating
grating
only
increasing grating salience
G
Motion responses
(Competing Stimuli)
optomotor (degrees/sec)
100
80
60
40
20
0
***
***
no
grating
grating
only
increasing grating salience

Figure 2

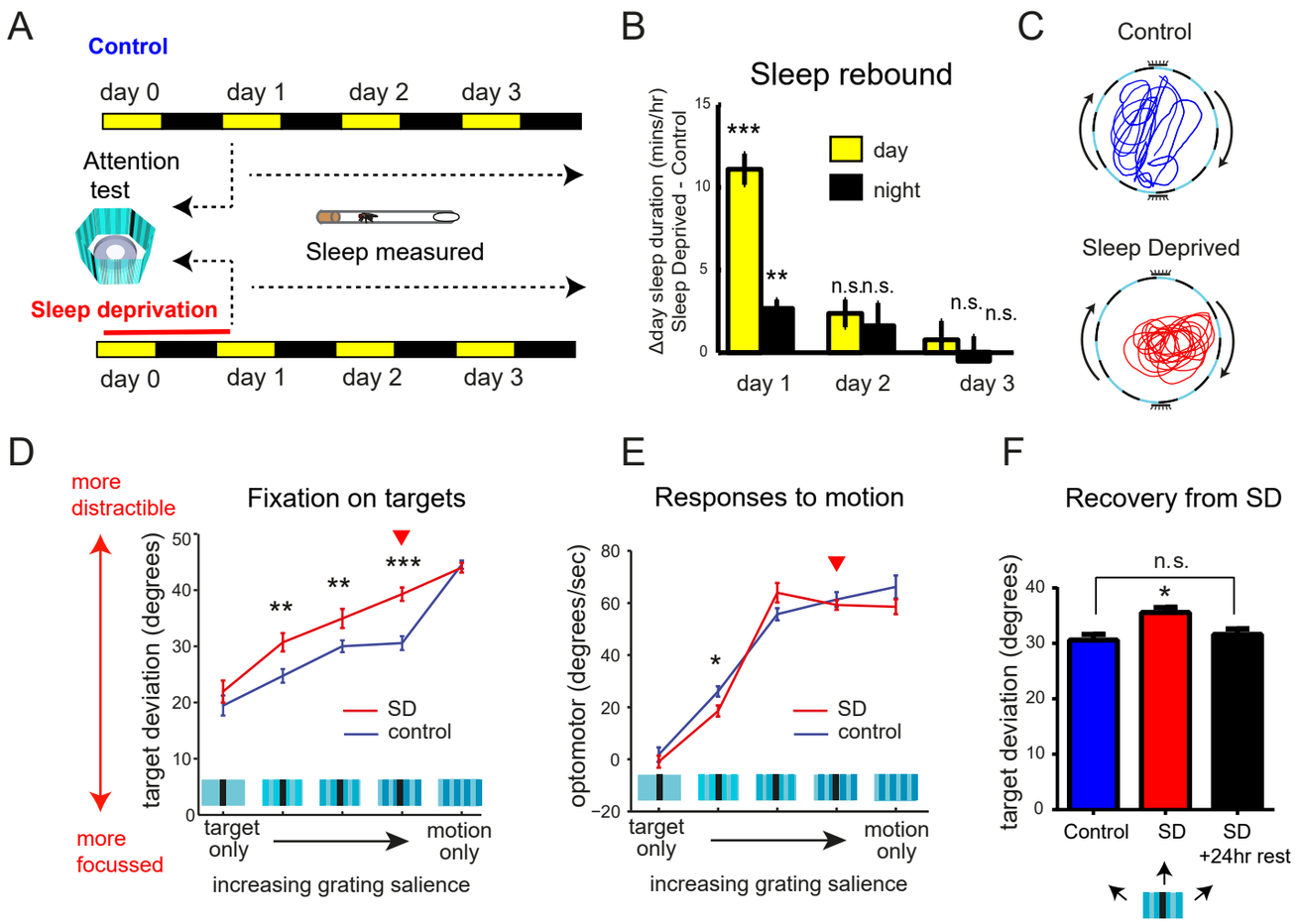

A
Control
day 0
day 1
day 2
day 3
Attention test
Sleep measured
Sleep deprivation
day 0
day 1
day 2
day 3
B
Sleep rebound
Δday sleep duration (mins/hr)
Sleep Deprived - Control
15
10
5
0
***
**
n.s.
n.s.
n.s.
n.s.
day
night
day 1
day 2
day 3
C
Control
Sleep Deprived
D
more distractible
more focussed
Fixation on targets
target deviation (degrees)
50
40
30
20
10
0
**
**
***
SD
control
target only
motion only
increasing grating salience
E
Responses to motion
optomotor (degrees/sec)
80
60
40
20
0
−20
*
SD
control
target only
motion only
increasing grating salience
F
Recovery from SD
target deviation (degrees)
40
30
20
10
0
n.s.
*
Control
SD
SD +24hr rest

Figure 3

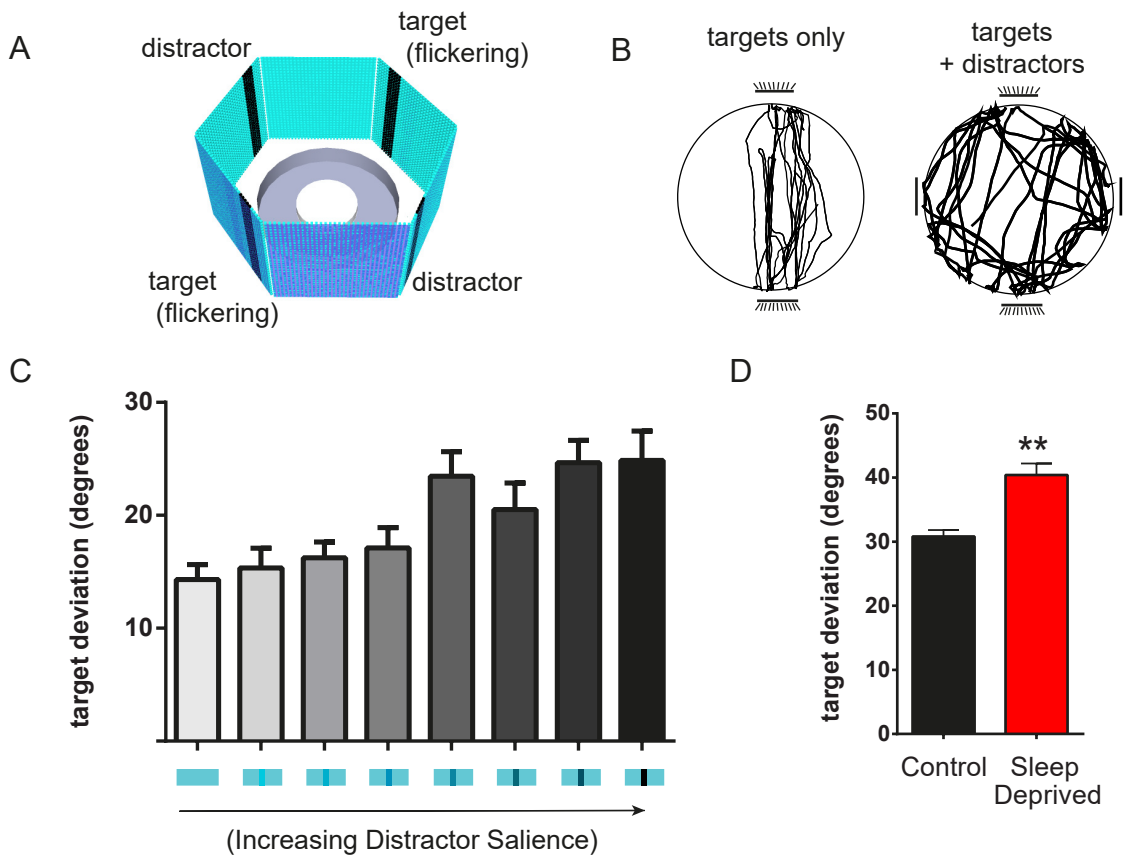

A
distractor
target
(flickering)
target
(flickering)
distractor
B
targets only
targets
+ distractors
C
target deviation (degrees)
30
20
10
(Increasing Distractor Salience)
D
target deviation (degrees)
50
40
30
20
10
0
**
Control
Sleep
Deprived

Figure 4

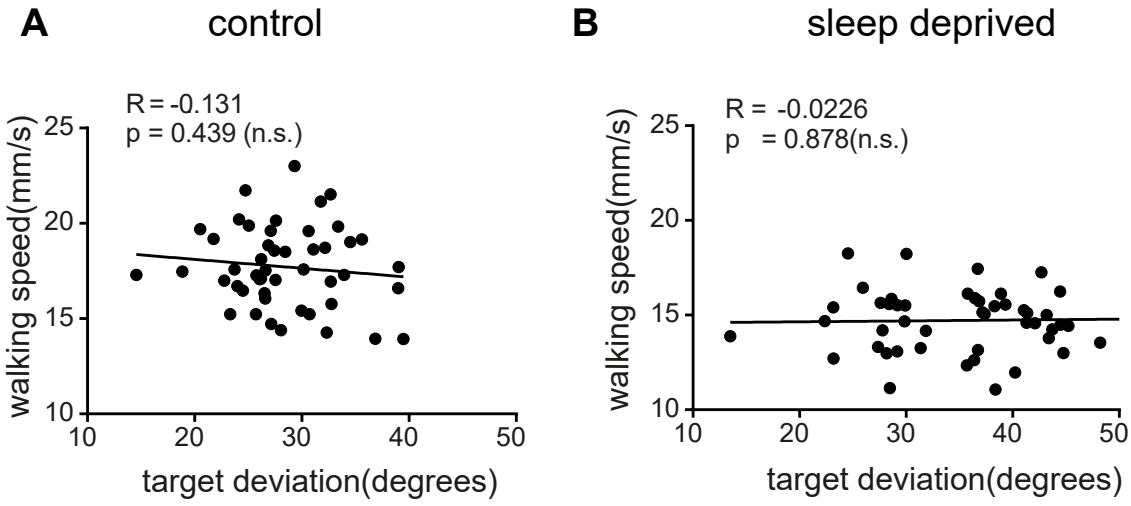

A
control
R = -0.131
p = 0.439 (n.s.)
walking speed(mm/s)
25
20
15
10
10
20
30
40
50
target deviation(degrees)
B
sleep deprived
R = -0.0226
p = 0.878(n.s.)
walking speed(mm/s)
25
20
15
10
10
20
30
40
50
target deviation(degrees)

Figure 5

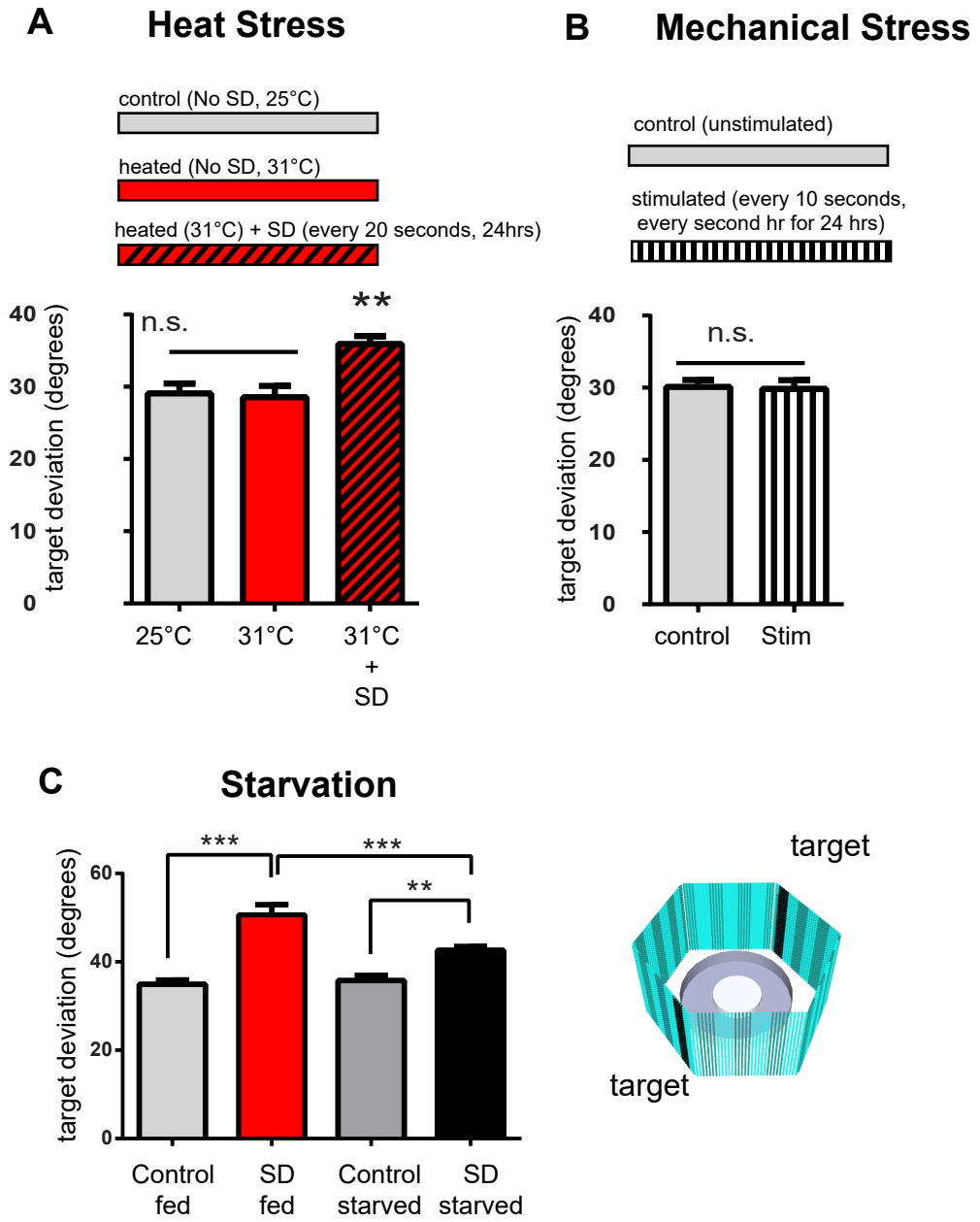

A
Heat Stress
control (No SD, 25°C)
heated (No SD, 31°C)
heated (31°C) + SD (every 20 seconds, 24hrs)
target deviation (degrees)
40
30
20
10
0
n.s.
**
25°C
31°C
31°C
+
SD
B
Mechanical Stress
control (unstimulated)
stimulated (every 10 seconds,
every second hr for 24 hrs)
target deviation (degrees)
40
30
20
10
0
n.s.
control
Stim
C
Starvation
target deviation (degrees)
60
40
20
0
***
***
**
Control
fed
SD
fed
Control
starved
SD
starved
target
target

Figure 6

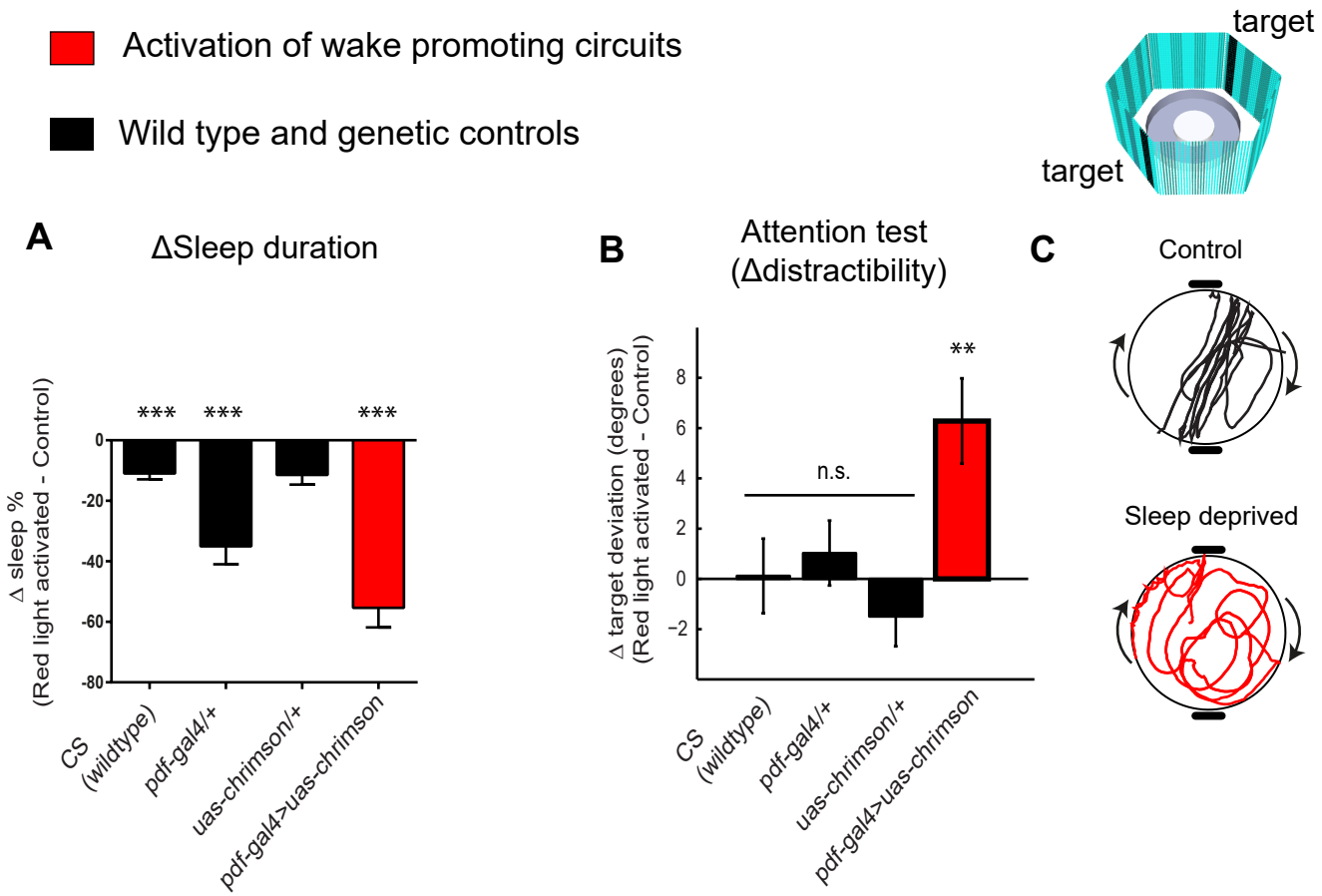

Activation of wake promoting circuits
Wild type and genetic controls
target
target
A
ΔSleep duration
Δ sleep %
(Red light activated - Control)
***
***
***
0
-20
-40
-60
-80
CS (wildtype)
pdf-gal4/+
uas-chrimson/+
pdf-gal4>uas-chrimson
B
Attention test
(Δdistractibility)
Δ target deviation (degrees)
(Red light activated - Control)
**
n.s.
8
6
4
2
0
−2
CS (wildtype)
pdf-gal4/+
uas-chrimson/+
pdf-gal4>uas-chrimson
C
Control
Sleep deprived

Figure 7

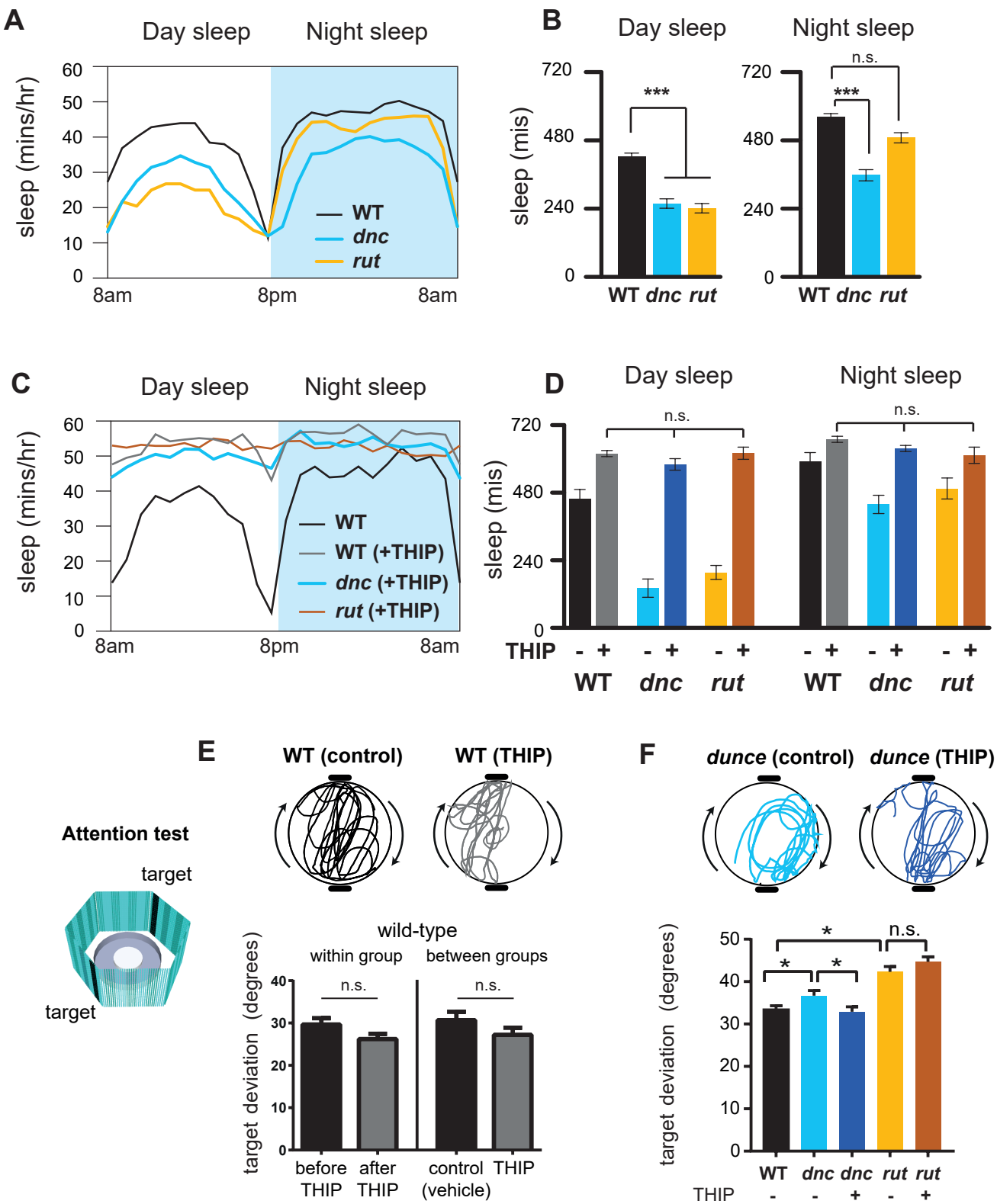

A
Day sleep
Night sleep
sleep (mins/hr)
60
50
40
30
20
10
0
8am
8pm
8am
WT
dnc
rut
B
Day sleep
Night sleep
sleep (mis)
720
480
240
0
***
n.s.
WT dnc rut
C
Day sleep
Night sleep
sleep (mins/hr)
WT
WT (+THIP)
dnc (+THIP)
rut (+THIP)
D
Day sleep
Night sleep
sleep (mis)
n.s.
THIP
- +
WT dnc rut
E
WT (control)
WT (THIP)
Attention test
target
target
wild-type
within group
between groups
target deviation (degrees)
n.s.
before THIP
after THIP
control (vehicle)
THIP
F
dunce (control)
dunce (THIP)
target deviation (degrees)
*
n.s.
WT dnc dnc rut rut
THIP
- - + - +

Figure S1

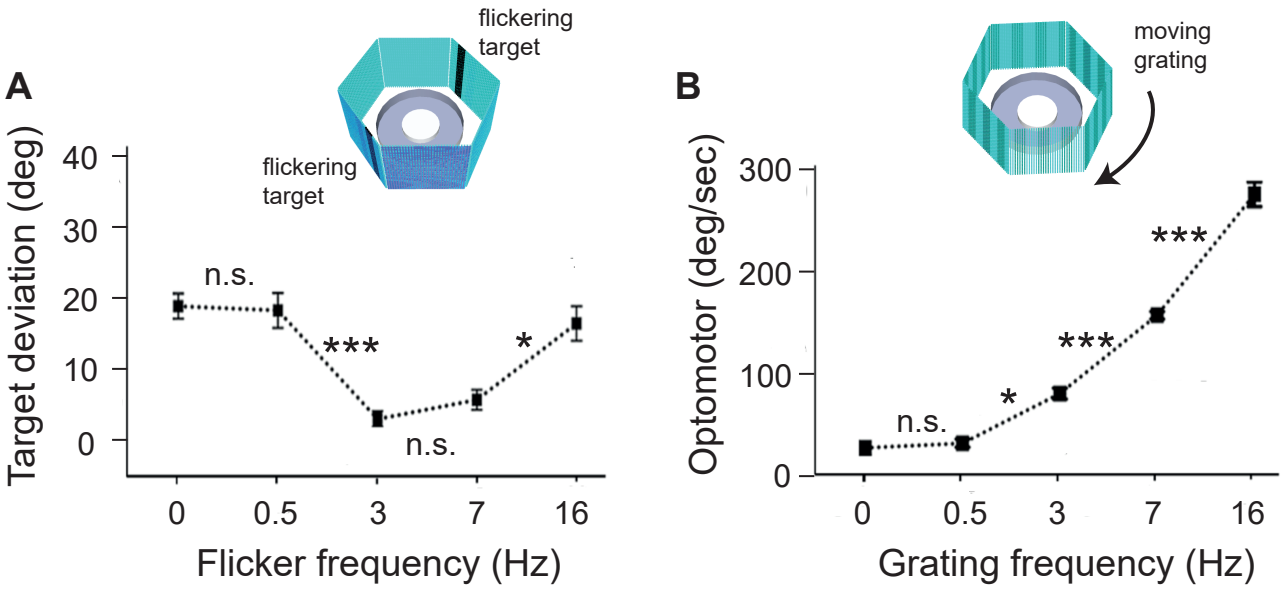
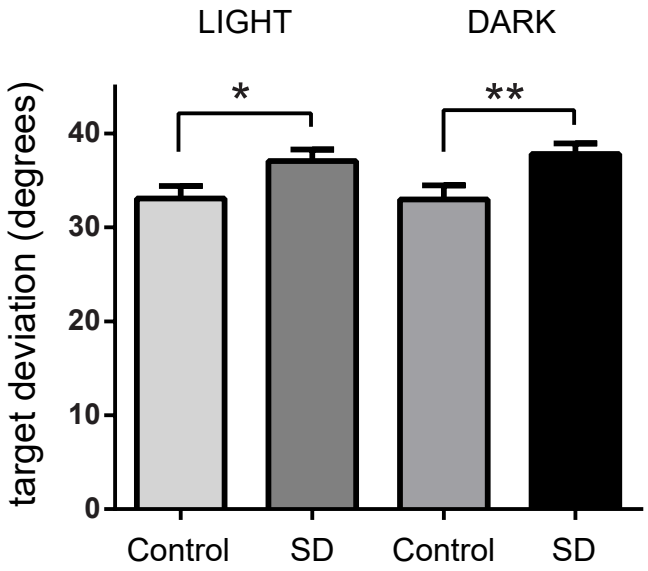
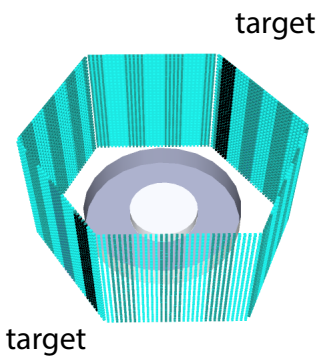

A
flickering target
flickering target
Target deviation (deg)
40
30
20
10
0
n.s.
***
n.s.
*
0
0.5
3
7
16
Flicker frequency (Hz)
B
moving grating
Optomotor (deg/sec)
300
200
100
0
n.s.
*
***
***
0
0.5
3
7
16
Grating frequency (Hz)

Figure S2


LIGHT
DARK
*
**
target deviation (degrees)
40
30
20
10
0
Control
SD
Control
SD


target
target