

RESEARCH ARTICLE

Sleep regulates visual selective attention in *Drosophila*

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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 optimizing 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 specifically improved attention in the learning mutant *dunce*. Our results reveal a key function of sleep in optimizing attention processes in *Drosophila*, and establish a behavioral paradigm that can be used to explore the molecular mechanisms involved.

KEY WORDS: Attention, Sleep, *Drosophila*, Flies, Behavior

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 behavior. 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 and Born, 2010) or by maintaining optimal levels of neuronal functions by globally altering synaptic strengths (Tononi and Cirelli, 2014). 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 could accrue when sleep homeostasis mechanisms are not in place (Kirszenblat and van Swinderen, 2015). Consistent with this idea, sleep deprivation in humans leaves basic sensory processing intact (Casagrande et al., 2006; Killgore, 2010; Kendall et al., 2006), whereas tasks that involve high attentional load are impaired (Chee

and Chuah, 2007; Kong et al., 2011). For this reason, sleep and attention may share a deeper relationship than previously thought, and one that has not been thoroughly investigated in genetic models such as *Drosophila*.

In *Drosophila*, sleep deprivation has been associated with deficits in a variety of behaviors. Sleep deprivation impacts olfactory and visual memory in *Drosophila* (Glou et al., 2012; Seugnet et al., 2008), as well as courtship memory (Ganguly-Fitzgerald et al., 2006) and aggression in male flies (Kayser et al., 2015). In contrast to sleep deprivation, induction of sleep has been shown to reverse learning deficits in the short-term memory mutants *dunce* and *rutabaga*, and in a fly model of Alzheimer's disease (Dissel et al., 2017). This suggests that sleep can be used as a powerful therapeutic to enhance learning and memory. As the ability to pay attention may be a prerequisite for the aforementioned behaviors, we questioned whether optimizing selective attention may be a key function of sleep in *Drosophila*.

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

MATERIALS AND METHODS

Fly stocks and experimental conditions


Strains used in this study were *Canton-S* (CS) wild-type flies, *dunce*¹ mutants (outcrossed to CS) and *rutabaga*²⁰⁸⁰. Flies were cultured on standard medium (agar, yeast, sugar, water, nipagen, propionic acid) at 25°C, 50–60% humidity and reared with a 12 h light:12 h dark cycle. The same conditions were used for sleep experiments.

Sleep and visual behavior

Our visual arena was adapted from Buridan's paradigm (Götz, 1980). Flies had their wings clipped under CO₂, at least 2 days prior to the experiment. During the experiment, flies walked freely on a round platform, 86 mm in diameter, surrounded by a water-filled moat to prevent escape (see Fig. S1). 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 min, 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 min 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

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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 *Drosophila* arousal tracking (DART) system, as previously described (Faville et al., 2015) using the 5 min 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, Shenzhen, China). For specifications of set-up, see Fig. S1. 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 (Straw et al., 2008), written in Python programming language (Ferguson et al., 2017), and are available upon request. The walls of the arena consisted of 6 LED panels of green (520 nm) and blue (468 nm) LEDs that formed a hexagon surrounding the moat (29 cm diameter, 16 cm height), and onto which the visual stimuli were presented. Fixation stimuli were two dark stripes 180 deg apart, each 9 deg in width and 45 deg in height from the centre of the arena. The fixation stripes ranged from 38 to 55 deg in height and 4–14 deg in width depending on the fly's position in the arena. For visual competition experiments, 7 Hz flickering stripes (targets) were overlaid on a 3 Hz grating (speed 54 deg s⁻¹, luminance 402 lx) 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 lx.

Sleep deprivation

Female CS flies had wings clipped on day 0–1 post-eclosion, and were then reared at low density in vials (20 females, and up to 5 males). When flies were 3–5 days old they were placed in fresh vials, and sleep deprived for 24 h from 11:00 h until 11:00 h the next day, and then tested in the visual arena immediately afterwards. Control flies were reared in exactly the same way (i.e. wings clipped at the same time, and then transferred to fresh vials), except they were not sleep deprived. Mechanical sleep deprivation was achieved using a sleep-nullifying apparatus (SNAP) device (inside a 25°C incubator), 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 20 s (or 10 s where indicated). Flies were contained in vials (20 females, 5 males) during the sleep deprivation, because containing them individually in small Trikinetics tubes affected their performance in the attention assay. During the experiments, flies waiting to be tested were gently handled at least every 3 min so that they could not fall asleep prior to testing. For correlation analysis between sleep and attention, flies were sleep deprived as a group in vials for 24 h, tested individually for visual attention, and then in the following 6 h, sleep was measured for each individual in Trikinetics tubes.

Pharmacology

THIP was administered to flies in standard food at 0.1 mg ml⁻¹ concentration for 2 days, and removed 1 h 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 deg s⁻¹) in the direction of the moving grating was calculated. CeTran (3.4) software (Colomb et al., 2012) was also used to calculate walking speed during visual experiments, and pausing (the number of times a fly was immobile for more than one second). 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 the normality of the data, and *t*-tests or one-way ANOVAs were used to detect significant differences between groups.

RESULTS

A visual attention paradigm for freely walking flies

Selective attention allows us to focus on a single object or group of objects, while ignoring less salient information (La Berge, 1983; Eriksen and St James, 1986). To examine attention-like behavior in freely walking *Drosophila*, we designed a paradigm to measure behavioral responses of flies to competing visual stimuli, involving 'targets' and 'distractors'. We took advantage of two robust visual behaviors in *Drosophila*: (1) fixation – a fly's tendency to orient and walk towards a visually salient object – and (2) optomotor behavior, whereby a fly will turn in the same direction as wide-field motion to stabilize its visual surroundings (Heisenberg and Wolf, 1984). Fixation has been previously measured using Buridan's paradigm, where flies with clipped wings walk back and forth between two opposing vertical black stripes (Götz, 1980; Ferguson et al., 2017). We modified this paradigm to measure behavioral responses to competing visual stimuli, using two opposing stripes as 'targets' in the foreground and adding a wide-field motion stimulus as the 'distractor' in the background (Fig. 1A). A similar configuration has been used for 'figure/ground' discrimination in tethered flight experiments (Heisenberg and Wolf, 1984; Fox et al., 2014; Fenk et al., 2014; Aptekar et al., 2015; Aptekar and Frye, 2013), but this complex stimulus has not been tested in walking flies.

We measured fixation on the targets by calculating the deviation angle between the fly's heading direction and the target stripe, with smaller angles indicating greater attention to the target (Fig. 1B) (Colomb et al., 2012). Response to the distractor, the motion stimulus, was measured by the angular velocity in the direction of motion [the turning angle s⁻¹ (γ): Fig. 1B]. First, we investigated visual responses to both stimuli alone, across a range of frequencies (Fig. S2A,B). Flies fixated best on targets flickering in the range of 3–7 Hz (Fig. S2A), while optomotor responses were highest at 16 Hz (Fig. S2B). For visual competition experiments, we used a target frequency of 7 Hz, as this stimulus was very salient to the flies and has previously been found to evoke strong fixation behavior (Paulk et al., 2015). The distractor was a lower-contrast grating that spanned the entire arena and moved 'behind' the flickering targets. We used a grating frequency of 3 Hz (54 deg s⁻¹) at a mid-luminance contrast, since we found responses under these conditions were robust and consistent among flies, eliciting circular walking responses (Fig. 1E), and provided a low enough level of distraction for the flies such that they could still continue to fixate on the targets. Adding this low-contrast motion distractor significantly reduced the flies' fixation on the target stripes: the straight paths evident when the targets were presented alone (Fig. 1C) were replaced by a combination of straight paths and circular paths, often angled in the direction of motion (Fig. 1D). We modified the salience of the grating distractor by adjusting the luminance contrast in linear increments (see Materials and

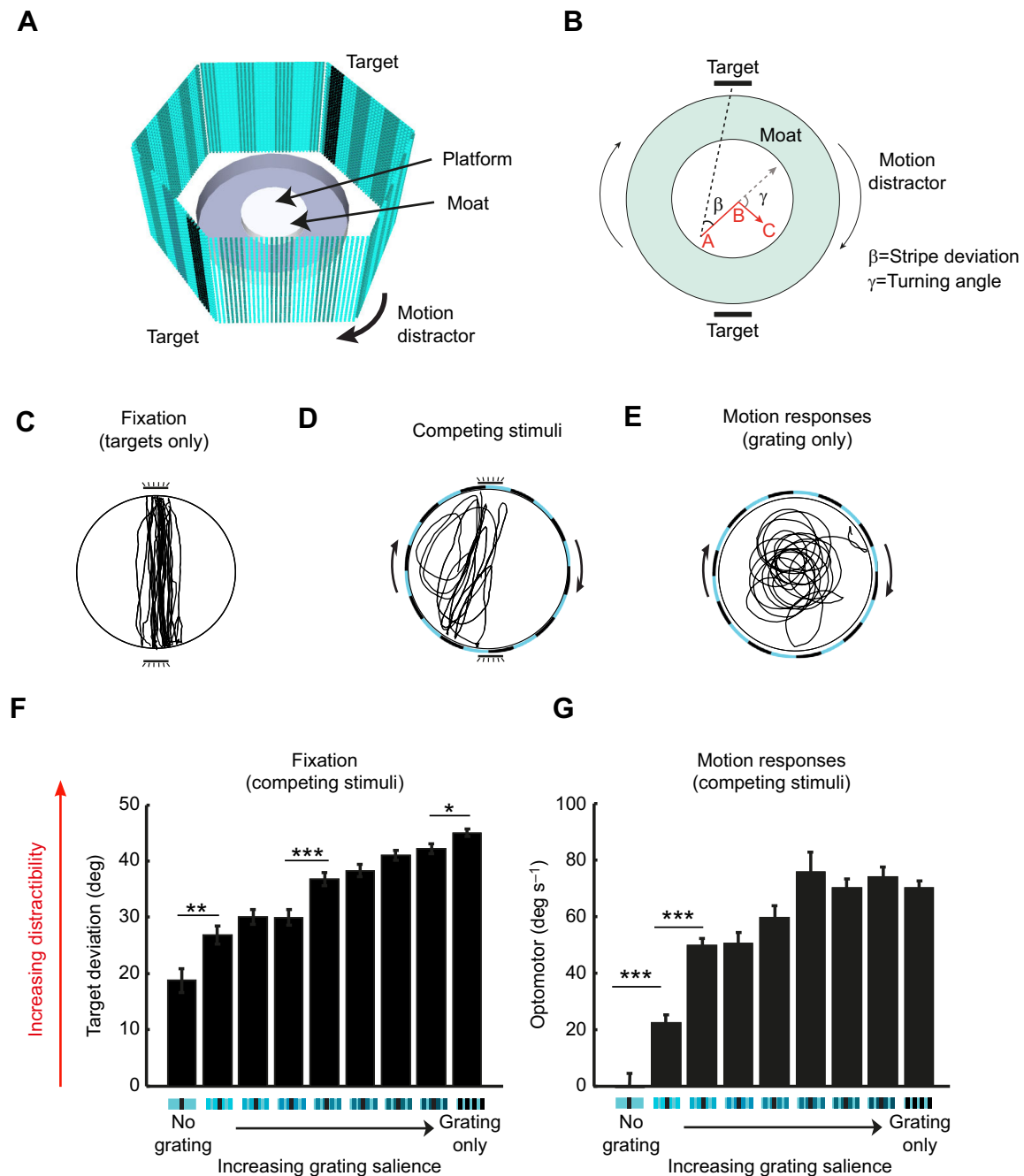


Fig. 1. A visual attention paradigm. (A) Diagram of experimental set-up. Flies walk on a platform surrounded by a moat of water, with six LED panels forming walls onto which visual cues are presented. Visual attention is measured by fixation on either of two opposing stripes ('fixation targets') in the presence of a motion stimulus ('distractor'). (B) Calculating fixation and optomotor responses (adapted from Colomb et al., 2012). Target fixation is measured by the stripe deviation (β), the angle between the fly's heading direction (e.g. red line from A to B) and the centre of the target stripe that is in the direction of movement (dotted black line). Response to the motion stimulus (optomotor) is determined by the fly's angular velocity, i.e. the fly's turning angle (γ) per second. (C–E) Example traces of a fly's walking path in response to flickering target stripes (C), competing stimuli (D) or a motion stimulus (E). (F,G) Quantification of target deviation (F) or optomotor response (G) in wild type Canton-S flies when different visual cues were presented: fixation targets alone ('no grating') or fixation targets combined with gratings of increasing salience (luminance contrast) or grating alone. $n=10$ flies for each condition, * $P<0.05$, ** $P<0.01$, *** $P<0.001$ by t -tests between adjacent conditions.

Methods) (Fig. 1F,G). As expected, when the grating had a higher contrast (more salient), the distraction effect increased (Fig. 1F). Motion responses to the distractor also increased (Fig. 1G) but appeared to plateau earlier than target deviation in response to increasing the distractor salience. This suggests that increased target deviation (Fig. 1F) may be a more sensitive measure of the distractor effect than increased optomotor behavior (Fig. 1G). In summary,

our results show that attention-like responses towards fixed targets can be effectively modulated (and titrated) by a motion distractor stimulus in freely walking flies.

Sleep-deprived flies are more distractible

Studies in humans and other animals have shown that sleep loss can impair a variety of cognitive behaviors, such as learning, memory

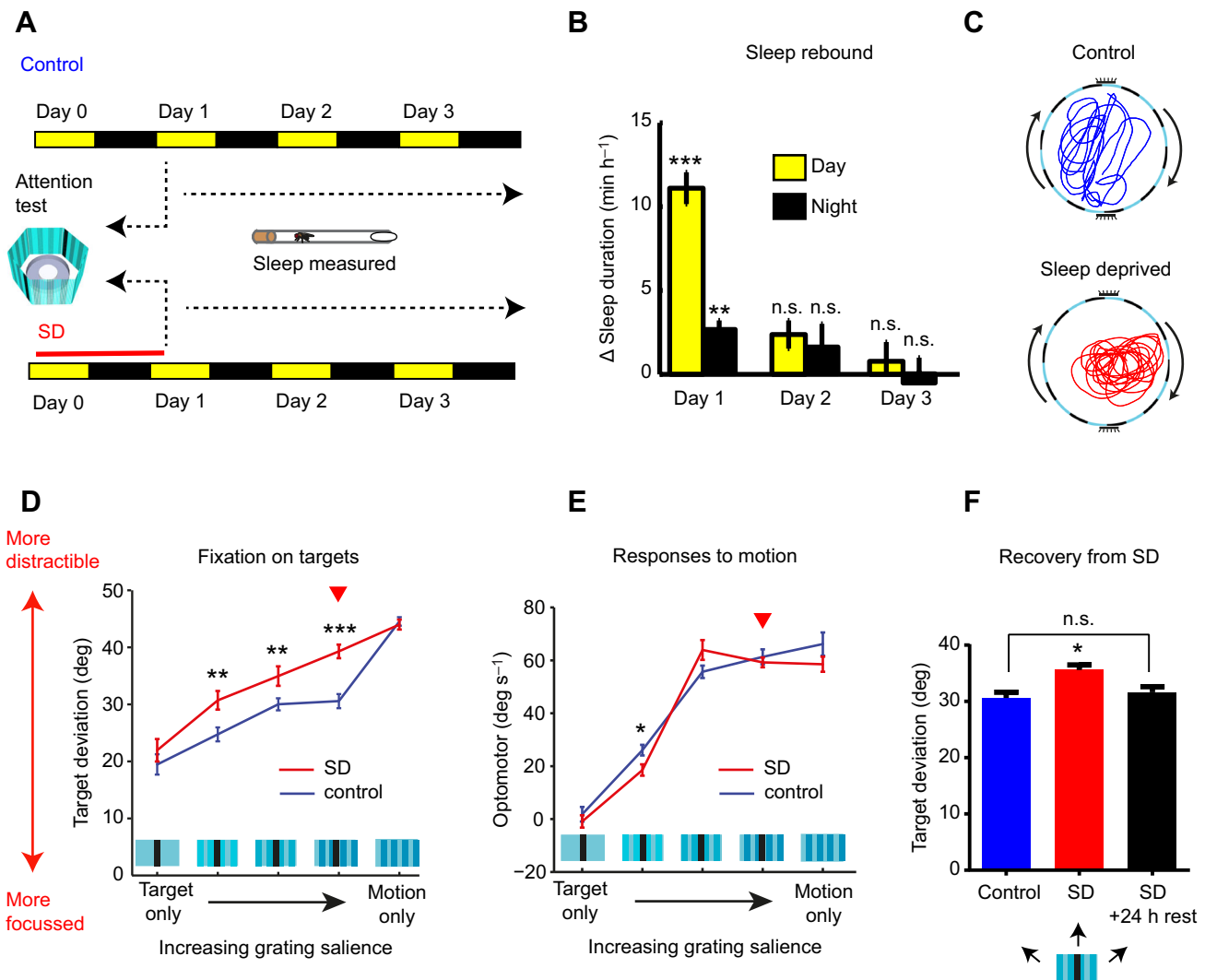


Fig. 2. Sleep deprivation makes flies more distractible. (A) Sleep deprivation protocol. Flies were sleep deprived for 24 h and visual attention was tested the following day (day 1), and compared with control (non-sleep-deprived flies). (B) Flies subjected to the sleep deprivation protocol in (A) showed a sleep rebound (increase in sleep) measured by the difference in sleep compared with control (non-sleep-deprived) flies. (C) Example traces of walking behavior in a control and sleep-deprived fly responding to competing visual cues. (D,E) Quantification of target deviation (D) or optomotor response (E) in control vs sleep-deprived (SD) flies responding to target objects alone (target only), competing visual cues (objects and grating together, with three levels of grating salience) and grating alone (motion only). (F) Attention returns to normal levels in flies rested for 24 h following sleep deprivation (tested with objects and mid-salience grating). $n=60$ flies in A,B; $n=16$ flies for each condition in D,E; $n>12$ flies in F. Red arrowheads indicate the grating contrast that was used for all subsequent experiments * $P<0.05$ (one-way ANOVA in F, t -test in E) and ** $P<0.01$, *** $P<0.001$ by t -test between control and sleep deprived; n.s., not significant. Error bars indicate s.e.m.

and attention (Alhola and Polo-kantola, 2007; Drummond et al., 2012; Lim and Dinges, 2010). Similarly, studies in *Drosophila* have indicated a role for sleep in visual and olfactory memory (Glou et al., 2012; Li et al., 2009; Seugnet et al., 2008; Seugnet et al., 2011). However, it is not known whether sleep modulates attention-like behavior in *Drosophila*. To investigate whether sleep deprivation affects visual attention, we sleep deprived flies for 24 h using the SNAP apparatus according to previous methods (Shaw et al., 2002). We first confirmed that flies were sleep deprived by examining their sleep rebound (i.e. the increase in sleep need following sleep deprivation). Following sleep deprivation, sleep was monitored using the DART system (Faville et al., 2015) across three consecutive days, while another group of flies was tested for visual attention (Fig. 2A). As expected, sleep-deprived flies slept more than control flies did on the day after sleep deprivation (day 1), particularly during the daytime; whereas by days 2 and 3, there was

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

Following sleep deprivation, we measured fixation to stationary targets and optomotor responses to a moving grating, in scenarios in which the stimuli were presented alone or in competition (with the grating at three different levels of luminance contrast of the distractor) (Fig. 2D,E). Optomotor responses to the moving grating in all visual scenarios were largely unaffected by sleep deprivation (Fig. 2E), as were fixation responses in the absence of the grating (Fig. 2D, first point from the left). Interestingly, the effect of sleep deprivation on fixation behavior was only evident when the two visual stimuli were combined (Fig. 2C), as indicated by significantly increased target deviation for all three combined conditions (Fig. 2D). This suggests that sleep-deprived flies are specifically affected in their response to visual competition. Finally, we tested whether flies could recover from sleep deprivation by allowing them

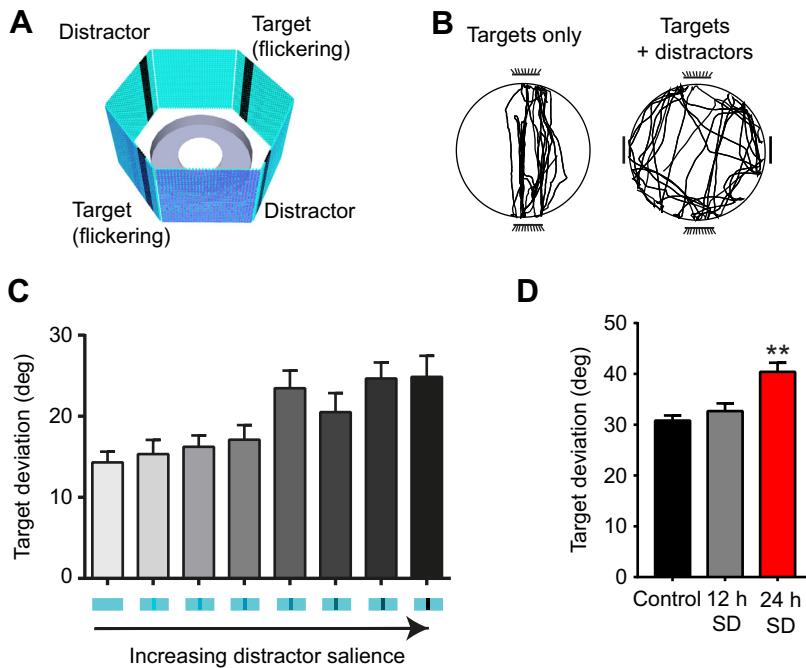


Fig. 3. Sleep deprivation increases distractibility to stationary objects. (A) Diagram of visual stimuli, with flickering target stripes and non-flickering distractor stripes. (B) Example traces of a fly responding to targets only, or targets with distractor stripes. (C) Target deviation scores with increasing distractor salience (luminance contrast to background). (D) Target deviation for controls and sleep-deprived flies (sleep deprived for 12 h at night, or 24 h across the day and the night) in the presence of distractor objects. $n=20$ flies in C; $n=30$ flies in D. $**P<0.0001$ by one-way ANOVA in D.

to rest for 24 h. For this experiment (and all subsequent experiments involving visual competition), we used a grating of mid-luminance contrast (indicated by the red arrowheads in Fig. 2D,E). Again, we found that sleep-deprived flies were more distractible (Fig. 2F, red bar), but their attention returned to normal following 24 h rest (Fig. 2F, black bar). Overall, our results suggest that sleep deprivation alters visual attention, and that this effect is reversible.

Although we saw no alterations to optomotor responses of sleep-deprived flies when the grating was presented alone, we considered the possibility that the sleep deprivation phenotype may be caused by an increased sensitivity to motion, which could potentially arise from the preceding 24 h of constant motion they experience in the SNAP apparatus. However, flies sleep deprived in 24 h of darkness showed a similar impairment of attention compared with controls, suggesting that visual experience did not play a role in the sleep deprivation phenotype (Fig. S3). We next asked whether the increased distractibility of sleep-deprived flies was specific to the motion stimulus, or whether it could apply to other types of visual distractors. We used a stationary stripe as a distractor that was identical to the target but less salient as it was non-flickering (Fig. 3A). As with the moving grating in the preceding experiments, attention to the target could be titrated by modulating the salience (i.e. contrast) of the distractor (Fig. 3B,C); increasing the salience of the distractor correspondingly increased distractibility (Fig. 3C). Interestingly, and consistent with our previous attention experiments, we found that sleep depriving flies for 24 h resulted in increased target deviation (poorer attention to the target) in the presence of this alternative distractor object (Fig. 3D, red bar). Our observation that sleep deprivation is similarly disrupted by different types of distractors suggests that a common attention mechanism is affected, which is not dependent on lower-level visual responses.

We next asked whether increased distractibility could be related to the amount of sleep lost. We submitted flies to a shorter period of sleep deprivation (12 h at night), and although there was a slight trend towards increased distractibility for the 12 h sleep deprivation, this effect was not significant (Fig. 3D, grey bar). This suggests that

24 h of sleep deprivation is required to observe effects on attention (Fig. 3D). We also wished to examine the amount of sleep loss in individual flies to see whether it was correlated with the severity of the attention defect in the same animals. In attempting this experiment, we made an interesting observation: flies that have been housed in small tubes used for sleep analysis (Trikinetics tubes), behaved erratically and were unable to fixate normally on visual objects (Fig. 4A,B). The effect on fixation behavior appeared to be due to confinement, not isolation, since flies that were isolated in larger vials behaved normally (Fig. 4A,B). Interestingly, their optomotor behavior appeared largely normal, indicating that the impairments were specific to fixation behavior (Fig. 4C). The abnormal fixation behavior of flies housed in sleep analysis tubes precluded us from addressing how individual attention behavior correlated with prior sleep. Nevertheless, we were able to measure sleep following the attention test in flies that had been sleep deprived in larger vials, as per our protocol (see Materials and Methods). We found no correlation between sleep and attention (Pearson r correlation, -0.041 ; $P=0.802$; $n=40$ flies), although how 'sleepy' individual flies actually were remained unknown. The effect of confinement on visual fixation (Fig. 4B) presents a challenging problem for relating sleep and attention phenotypes at an individual level.

Given that sleep deprivation reduces overall activity, we wondered whether this may explain the reduced orientation to salient features in the visual attention assay. To investigate this, we performed a correlation analysis on all data (from Fig. 2), to see if walking speed of individual flies could predict selective attention behavior (target deviation). However, we found no correlation between attention and walking speed for either sleep-deprived or control groups (Fig. 5A,B).

Considering that the sleep deprivation protocol might be stressful (it involves waking the flies every 20 s by mechanical stimulation), we further examined the effects of different types of stress on the flies' visual attention. This allowed us to answer two questions: (1) whether attention phenotypes could be modulated by environmental stressors (e.g. heat, mechanical stress and starvation), which would indicate that the sleep deprivation effect on attention may simply be

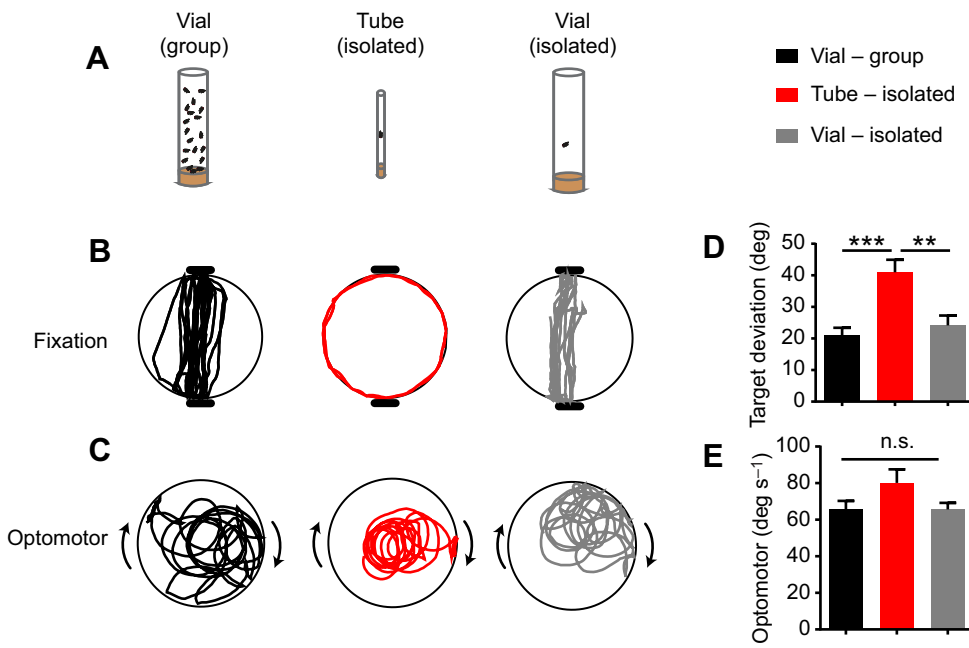


Fig. 4. Visual fixation is reduced following confinement in Trikinetics (sleep-analysis) tubes. (A) Fixation and optomotor phenotypes were examined following different housing conditions: social rearing in vials, isolation in Trikinetics tubes or isolation in vials. (B,C) Representative walking paths for individual flies for fixation (B) and optomotor responses (C). (D) Stripe deviation is increased in flies housed in Trikinetics tubes. (E) Optomotor responses were similar across the different housing conditions. $n=10$ flies per group. ** $P<0.01$, *** $P<0.001$, one-way ANOVA (D) and Kruskal–Wallis multiple comparisons (E); n.s., not significant. Error bars indicate s.e.m.

caused by stressful situations, and (2) whether the sleep deprivation phenotype occurs under different environmental conditions (i.e. how robust the effect is). For all these experiments, we measured the flies' visual responses in the combined stimulus paradigm (in which flies fixate on targets in the presence of distractors) (Fig. 1A).

First, we measured the effect of heating flies to 31°C for 24 h (Fig. 6A). Prolonged exposure to heat had no effect on attention, whereas the increase in distractibility caused by sleep deprivation was still present under conditions of heat stress (Fig. 6A). This indicates that heat does not influence attention in this paradigm, or the effect of sleep deprivation on attention. Next, we measured the effect of mechanical stress, using a stimulation protocol that would provide the same total amount of stimulation experienced during the sleep deprivation protocol, but including extended unstimulated periods to allow sleep. This was achieved by subjecting flies to mechanical stimulation for 1 h followed by rest for 1 h, repeated across 24 h, but with double the rate of stimulation (to keep the actual number of stimuli the same as for the sleep-deprivation regime) (Fig. 6B). Unlike sleep-deprived flies, flies subjected to this stimulation regime containing rest periods had normal attention, indicating that the sleep deprivation effect was not due to mechanical stress of perturbing the flies (Fig. 6B). We next

assessed the effects of starvation, as sleep is known to be affected by food availability (Siegel et al., 2009). In *Drosophila*, food deprivation has been found to suppress sleep (Keene et al., 2010). Feeding was assessed during sleep deprivation by measuring the intake of food containing blue food dye and, as previously reported, sleep-deprived flies showed similar food intake to controls (Thimgan et al., 2010). We then tested whether depriving flies of food, with or without sleep deprivation, affected visual attention. The visual attention scores of starved controls were not different from those of fed controls, suggesting that starvation per se does not impair visual attention (Fig. 6C). As expected, sleep deprivation impaired visual attention in both starved and fed conditions but, interestingly, starvation was able to partially suppress this effect (Fig. 6C, 'SD fed' vs 'SD starved'), suggesting that starvation may provide some level of protection against the detrimental effects of sleep deprivation. In summary, our results show that sleep deprivation consistently affects visual attention, even under conditions of heat and starvation, but that visual attention is resistant to the effects of a variety of stressors in the absence of sleep deprivation. Therefore, sleep seems more important than lack of stress for maintaining optimal levels of visual attention.

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

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

We first quantified sleep in *dunce* and *rutabaga* mutants, and found that both these mutants slept significantly less than wild-type flies, with *dunce* flies sleeping less during the day and night, and

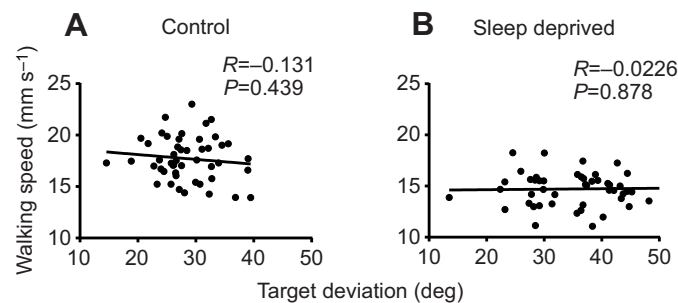


Fig. 5. Target deviation in the attention paradigm is not correlated with walking speed or sleep following sleep deprivation. (A,B) Correlation of walking speed and target deviation in control (A) and sleep-deprived (B) flies. $n=48$ flies. Pearson's correlation coefficients (R) were not significant (shown on graphs, alongside P -values), indicating that no correlations were found.

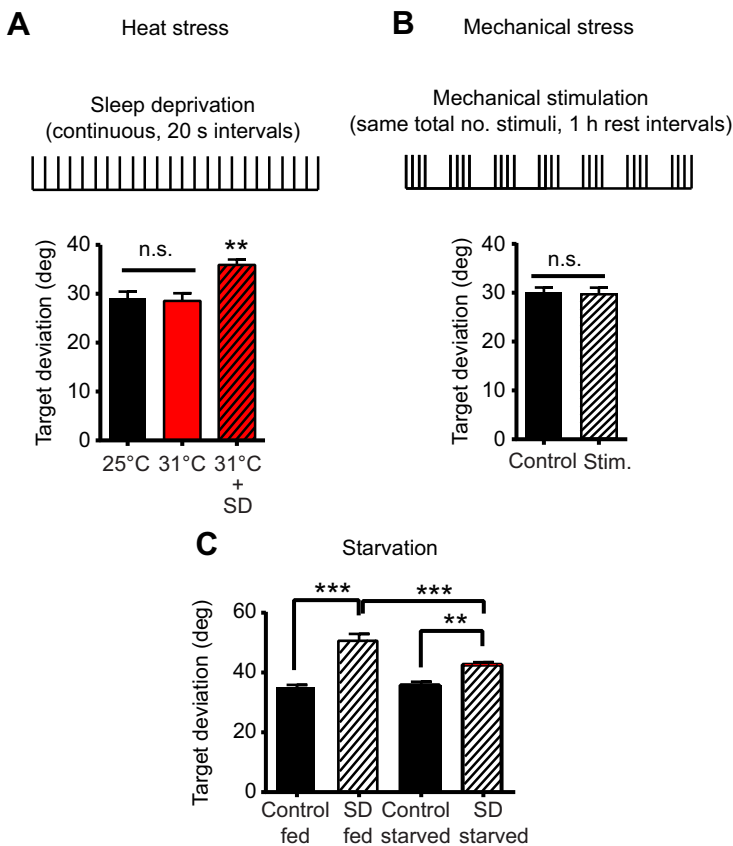


Fig. 6. Starvation, heat and mechanical stress effects on visual attention. Visual attention was measured by orientation towards 7 Hz flickering object ('targets') with a mid-luminance contrast grating distractor (see Materials and Methods). (A) Target deviation in flies that underwent heat stress (31°C) for 24 h with or without sleep deprivation. (B) Target deviation following 24 h mechanical stimulation. Mechanical stimulation delivered the same total number of stimuli as the sleep deprivation protocol, except with alternating 1 h periods of double frequency stimulation followed by rest (no stimuli). (C) Target deviation in flies that were starved and/or sleep deprived for 24 h. 'Control fed' vs 'control starved' groups were not significantly different. $n=17$ flies in A; $n=28$ flies in B; $n=20$ flies in C. ** $P<0.01$, *** $P<0.001$ by one-way ANOVA; n.s., not significant. Error bars indicate s.e.m.

rutabaga mutants sleeping less during the day (Fig. 7A,B). THIP could then be effectively used to increase sleep in both wild-type and mutant flies to similar levels (Fig. 7C,D). We next tested whether inducing sleep affected the visual attention of wild-type flies in our free-walking paradigm. THIP was fed to wild-type (CS) flies for 2 days and removed 1 h prior to testing their behavior (the same procedure used by Dissel et al., 2015). We performed a within-group experiment (the same flies tested before and after induced sleep) and a between-group experiment (aged-matched flies, with or without induced sleep). In wild-type flies we observed that distractibility (deviation away from the targets) decreased slightly for those flies that had been induced to sleep more, in both experiments; however, this effect was not significant (Fig. 7E, $P=0.084$ and $P=0.187$ for within group and between group comparisons, by *t*-test).

We next tested whether increasing sleep altered attention in *dunce* and *rutabaga* mutants. Under normal conditions (without increasing sleep), *dunce* and *rutabaga* flies were significantly less attentive towards the visual targets compared with wild-type flies, as measured by increased target deviation (Fig. 7F, black bar compared with light blue and yellow bars). Interestingly, increased sleep following THIP administration was able to significantly reduce target deviation in *dunce* mutants but not *rutabaga* mutants (Fig. 7F), such that attention in *dunce* mutants with induced sleep was not different from that of wild-type flies (Fig. 7F, dark blue bar compared with black bar). Although THIP-induced sleep rescued attention, it did not significantly alter other behaviors during the experiment, including walking speed (Fig. S4A) and pausing (Fig. S4B). Crucially, simple fixation and optomotor behaviors were unchanged in THIP- versus non-THIP-treated *dunce* mutants (Fig. S4C,D). Overall, our data suggest that increasing sleep may improve attention in some learning mutants but not others, but does

not affect other behaviors related to locomotion or simple visual responses.

DISCUSSION

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

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

One might still ask whether sleep has a privileged role in regulating selective attention relative to other higher-order cognitive functions. Sleep loss is known to affect a variety of complex cognitive behaviors in humans, such as learning and memory,

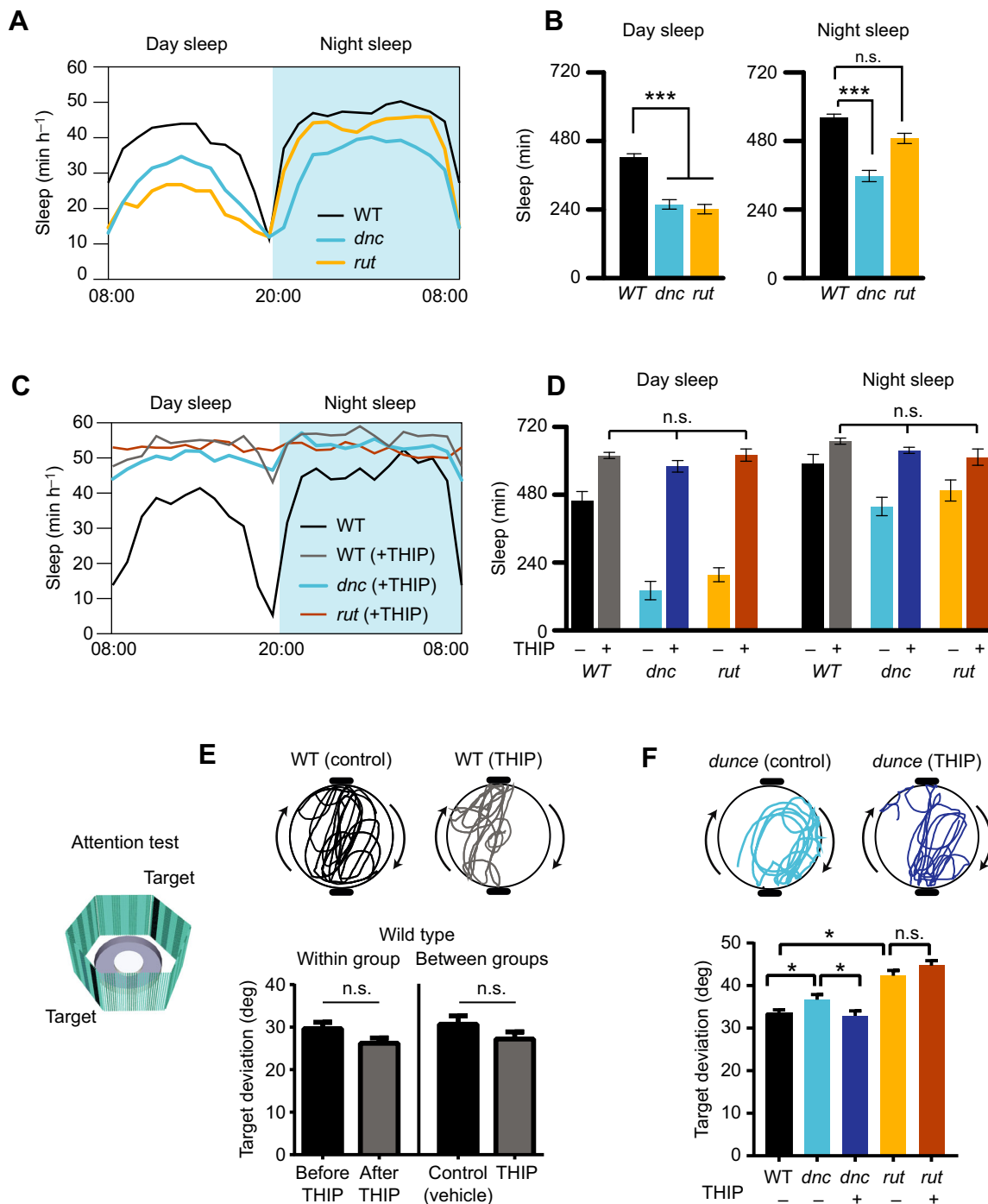


Fig. 7. Increasing sleep improves attention in *dunce* mutants. (A) Hourly sleep profiles averaged across 24 h for wild-type (Canton-S), *dunce* and *rutabaga* flies ($n=50$ flies). (B) Quantification of sleep duration averaged across the day and the night, from data in A. (C) Sleep across 24 h in wild-type flies, *dunce* (*dnc*) and *rutabaga* (*rut*) mutants treated with the sleep-promoting agent, THIP. Wild-type sleep without THIP is shown for comparison (black trace). $n=20$ flies. (D) Quantification of daytime and night-time sleep in wild-type, *dunce* and *rutabaga* flies with and without THIP; $n=20$ flies. (E) Target deviation of wild-type (Canton-S) flies on control (vehicle) or THIP (0.1 mg ml⁻¹) food. THIP was administered for 2 days and flies were removed from THIP 1 h prior to the visual attention test. 'Within group' compares flies before and after THIP administration ($n=11$ flies) and 'between group' compares age-matched flies fed control (vehicle) or THIP food ($n=8$ flies). (F) Stripe deviation in *dunce* mutants before and after 2 days of THIP ($n=30$ for *dunce* and CS, $n=16$ for *rutabaga*). * $P<0.05$, *** $P<0.0001$ by one-way ANOVA (A–D) and t -test (E,F); n.s., not significant. Error bars show s.e.m.

creative thinking, and even the ability to speak clearly or to appreciate humour (Harrison and Home, 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 that 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 stressors. 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). Another possibility is that starvation does not protect against sleep deprivation per se, but may inhibit sleep need such that optimal attention processes can be maintained in order to find food effectively. Indeed, *foraging* mutants of *Drosophila*, which tend to explore further for food, also appear to have a reduced need for sleep and are resistant to memory impairments caused by sleep loss (Donlea et al., 2012), whereas mutants selected for starvation resistance exhibit increased sleep (Masek et al., 2014). 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 behavior (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 normalize the attention of *dunce* mutants, but did not affect wild-type flies or *rutabaga* 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 et al., 2015). One interpretation is that attention is already optimal in wild-type *Drosophila*. In contrast, both *rutabaga* and *dunce* mutants have previously been identified as having attention deficits (van Swinderen, 2007), and our result confirmed this finding in a free-walking attention paradigm. However, it is not clear why inducing sleep would improve attention specifically in *dunce* mutants, and not in *rutabaga* mutants. It is possible that this may relate to their different sleep phenotypes – *dunce* mutants appear more severely sleep-deficient in our DART system (including at night, which *rutabaga* mutants are not) meaning that THIP had a greater ability to restore sleep to *dunce* mutants. Related to this, it is possible that the attention deficits of these mutants are due to different underlying causes. For example, the mutants may have poor attention because they sleep less, or they may sleep less because they have poor attention. This remains unresolved.

How inducing sleep could improve attention, or other aspects of cognition such as learning and memory, also remains unclear. There is some evidence that the sleep-promoting agent THIP can interact with specific GABA receptors to promote sleep in flies, via known sleep-promoting neurons of the dorsal fan-shaped body (Dissel et al., 2015), but can also modulate dopaminergic pathways to facilitate memories (Berry et al., 2015). Interestingly, a recent study found that increasing sleep promoted survival of wild-type flies exposed to oxidative stress (Hill et al., 2018). Whether increasing sleep in flies improves brain function through specific circuits, or by general cellular mechanisms such as reducing reactive oxygen species (ROS) needs to be further investigated. Our study opens up an

opportunity to understand the molecules, circuits and environmental factors involved in the role of sleep in optimizing attention.

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Competing interests

The authors declare no competing or financial interests.

Author contributions

Conceptualization: L.K., Y.Z., P.J.S., B.v.S.; Methodology: L.K., D.E., Y.Z., P.J.S., B.v.S.; Software: L.K., Y.Z., B.v.S.; Validation: L.K., D.E., B.v.S.; Formal analysis: L.K., D.E., J.G., Y.Z., B.v.S.; Investigation: L.K., D.E., J.G., Y.Z., B.v.S.; Resources: L.K., D.E., Y.Z., P.J.S., B.v.S.; Data curation: L.K., D.E., J.G., B.v.S.; Writing - original draft: L.K., B.v.S.; Writing - review & editing: L.K., D.E., P.J.S., B.v.S.; Visualization: L.K., D.E., J.G., B.v.S.; Supervision: P.J.S., B.v.S.; Project administration: L.K., P.J.S., B.v.S.; Funding acquisition: P.S., B.v.S.

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Data availability

All data and code used for this study are available upon request.

Supplementary information

Supplementary information available online at <http://jeb.biologists.org/lookup/doi/10.1242/jeb.191429.supplemental>

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