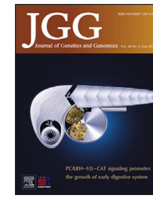
Journal of Genetics and Genomics 44 (2017) 319–326

Contents lists available at ScienceDirect

Journal of Genetics and Genomics

Journal homepage: www.journals.elsevier.com/journal-of-genetics-and-genomics/



Original research

Increased food intake after starvation enhances sleep in *Drosophila melanogaster*

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A R T I C L E   I N F O

Article history:
Received 29 December 2016
Received in revised form
21 April 2017
Accepted 16 May 2017
Available online 13 June 2017

Keywords:
Drosophila
Feeding
Sleep
Starvation
Satiety

A B S T R A C T

Feeding and sleep are highly conserved, interconnected behaviors essential for survival. Starvation has been shown to potently suppress sleep across species; however, whether satiety promotes sleep is still unclear. Here we use the fruit fly, *Drosophila melanogaster*, as a model organism to address the interaction between feeding and sleep. We first monitored the sleep of flies that had been starved for 24 h and found that sleep amount increased in the first 4 h after flies were given food. Increased sleep after starvation was due to an increase in sleep bout number and average sleep bout length. Mutants of *translin* or *adipokinetic hormone*, which fail to suppress sleep during starvation, still exhibited a sleep increase after starvation, suggesting that sleep increase after starvation is not a consequence of sleep loss during starvation. We also found that feeding activity and food consumption were higher in the first 10–30 min after starvation. Restricting food consumption in starved flies to 30 min was sufficient to increase sleep for 1 h. Although flies ingested a comparable amount of food at differing sucrose concentrations, sleep increase after starvation on a lower sucrose concentration was undetectable. Taken together, our results suggest that increased food intake after starvation enhances sleep and reveals a novel relationship between feeding and sleep.

1. Introduction

Organisms maintain homeostasis by tightly regulating numerous biological processes, such as feeding and sleep. Impairments in feeding or sleep are indicators of an organism's overall health. Poor sleep quality, high sleep loss, and high predisposition to sleep disorders are major symptoms in obesity (Romero-Corral et al., 2010; Beccuti and Pannain, 2011). In addition, chronic partial sleep deprivation can lead to an increased risk of obesity and diabetes (Sharma and Kavuru, 2010). There is also evidence that suggests an acute interaction between feeding and sleep. During periods of starvation, time spent in rapid eye movement (REM) sleep is significantly decreased, suggesting that food deprivation alters sleep architecture (MacFadyen et al., 1973). Furthermore, sleep deprivation for limited time periods (a couple of nights) has the ability to alter feelings of hunger and levels of feeding-related hormones (Knutson et al., 2007). Despite substantial evidence of the interaction between feeding and sleep, little is known about the mechanisms that link these two behaviors.

The fruit fly, *Drosophila melanogaster*, displays several behavioral hallmarks of sleep and feeding, making it an excellent model organism for studying the genetic and neural regulation of these behaviors (Shaw et al., 2000; Hendricks et al., 2000; Sehgal and Mignot, 2011; Itskov et al., 2014). Flies have been increasingly used to study how sleep and feeding interact. For example, during periods of starvation, flies increase their activity and decrease sleep amount (Lee and Park, 2004; Keene et al., 2010; Thimgan et al., 2010). Mutations in core circadian clock genes, *clock* and *cycle*, have been shown to amplify starvation-induced sleep suppression, while mutations in *translin* (*trsn*), encoding an RNA-DNA binding protein, result in a loss of starvation-induced sleep suppression (Keene et al., 2010; Murakami et al., 2016). Mutations in *adipokinetic hormone* (*akh*), the insect analog of *glucagon*, have been shown to affect starvation-induced hyperactivity (Lee and Park, 2004). The role of *akh* in starvation-induced sleep suppression, however, remains unknown.

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http://dx.doi.org/10.1016/j.jgg.2017.05.006

Despite studies on the interaction between starvation and sleep, little is known about the mechanisms by which food intake, especially in high amounts, affect behavior. During periods of high metabolic needs, such as starvation, animals ingest a large amount of food to achieve nutritional homeostasis (Davis and Levine, 1977; Itskov et al., 2014; Yapici et al., 2016). Thus, increased food intake is mediated by prolonged periods of starvation. Increased food intake results in a series of behaviors among different animal species, such as in cessation of food ingestion (Antin et al., 1975; You et al., 2008). In humans, a state of drowsiness after a meal (postprandial somnolence) has been shown to be highly variable across individuals (Stahl et al., 1983). While studies have displayed a link between postprandial sleep and meal size/composition (Danguir and Nicolaidis, 1979; Danguir, 1987; Murphy et al., 2016), the numerous variable characteristics of postprandial sleep have limited the thorough analysis of its mechanistic basis.

To study the effect of increased food intake after starvation on sleep, our current study utilizes starvation to induce food intake and monitors sleep changes in *D. melanogaster*. We find that after starvation, sleep is profoundly increased for several hours. Furthermore, sleep increase after starvation is not due to sleep loss during starvation. The increased amount of feeding activity and food consumption in the first 30 min after starvation is sufficient to acutely increase sleep. Interestingly, low concentration of sucrose after starvation was not sufficient to increase sleep even though flies ingest an increased amount of food after starvation. Thus, our findings present new evidence to suggest an interaction between feeding and sleep which will lead to new investigations into the mechanisms that promote this interaction.

2. Results

2.1. *Drosophila* increase sleep after starvation

Although several studies have shown that starvation decreases sleep, the changes in sleep after starvation remain ambiguous (Keene et al., 2010; Thimgan et al., 2010). To address this question, we developed a paradigm to starve individual flies in activity tubes for 24 h and then allow them to feed again while continuously monitoring their sleep with the *Drosophila* Activity Monitoring System (DAMS) (Fig. 1A) (Pfeiffenberger et al., 2010). After a day of baseline sleep recording on sucrose and agar medium for food, we starved a group of wild-type flies for 24 h by providing them with only non-nutritious agar and compared their sleep with a group of wild-type flies fed *ad libitum* (“Fed *ad lib*”; Fig. 1A). Consistent with previous studies, sleep was dramatically decreased in female and male flies in the starved group (Figs. 1B, 1C, S1A and S1B) (Keene et al., 2010; Thimgan et al., 2010). This finding was conserved across several different wild-type backgrounds (Figs. 1C and S1B). After 24 h of starvation, flies were transferred back onto sucrose and agar medium to feed again (Fig. 1A). Sleep was significantly increased in the first 4 h after feeding in female and male flies in the starved > fed group (Figs. 1D 1E, S1C and S1D). This increase in sleep after starvation was conserved across other wild-type backgrounds (Figs. 1E and S1D). To address what sleep parameters were altered to increase sleep after starvation, we analyzed sleep bout number and average sleep bout length. We found that the sleep bout number and average sleep bout length were increased after starvation in female and male flies (Figs. 1F, 1G, S1E and S1F). We also performed high-resolution video recordings in the first 4 h after feeding to exclude periods of body movements such as grooming (Fig. S2). We found a similar fold increase in the starved > fed group compared to fed *ad lib* group as our DAMS recordings, suggesting that sleep increase after starvation is not due to feeding or grooming events (Fig. S2).

Supplementary video related to this article can be found at http://dx.doi.org/10.1016/j.jgg.2017.05.006.

2.2. Sleep increase after starvation does not depend on sleep loss during starvation

After sleep deprivation, animals use ill-defined homeostatic strategies to recover from sleep loss by increasing their sleep (Borbély, 1982). Since animals suppress sleep during starvation, sleep increase after starvation might be due to a homeostatic strategy to recover from sleep loss. To test this hypothesis, we studied fly mutants, which fail to suppress sleep during starvation. Previous studies have identified the *akh* gene, the fly ortholog of *glucagon*, as a crucial mediator of starvation-induced hyperactivity (Lee and Park, 2004). However, *akh*'s role in starvation-induced sleep suppression is unknown. We tested *akh* mutants for sleep changes during starvation and found that, unlike their wild-type controls, they do not suppress sleep (Fig. 2A and C). Remarkably, *akh* mutants continue to increase sleep after starvation (Fig. 2A and D). These results suggest that the sleep increase in flies after starvation is independent of previous sleep loss.

A recent study has shown that *trsn* fly mutants fail to show starvation-induced sleep suppression while preserving homeostatic feeding strategies, such as increased food intake, after starvation (Murakami et al., 2016). Thus, we used the *trsn* mutants to further test whether the sleep increase after starvation might be due to sleep loss during starvation. In support of the study of Murakami et al. (2016), we found that *trsn* mutants, unlike their wild-type controls, fail to decrease their sleep during starvation (Fig. 2B and C). After starvation, *trsn* mutants show an increase in sleep similar to wild-type controls (Fig. 2B and D). Together with *akh* mutants, these findings suggest that sleep increase after starvation does not depend on sleep loss during starvation.

Since *akh* has been associated with mediating starvation-induced hyperactivity, we tested the activity of *akh* mutants under fed *ad lib* conditions (Lee and Park, 2004). We found that *akh* mutants have a lower activity index (average beam crosses per waking minute) (Fig. 2E). Since *akh* mutants do not suppress sleep during starvation and have lower overall activity, *akh* may play a role in the connection between sleep and activity.

2.3. Starved flies acutely increase feeding for 30 min

Since sleep loss during starvation is not the sole driver of sleep increase after starvation, we examined whether fly feeding behavior after starvation regulates sleep. We video recorded individual wild-type flies in activity tubes after 24-h starvation, and tracked the percentage of flies feeding after starvation (see Materials and methods). The percentage of flies feeding was increased in starved flies within the first 30 min. After 30 min, this proportion decreased to match control levels, suggesting that a high amount of feeding behavior occurs in the first 30 min after starvation. (Fig. 3A and C).

To determine if homeostatic feeding in the first 30 min was sufficient to promote a satiated feeding state, we quantified food intake per fly via the Capillary Feeding (CAFE) assay (Ja et al., 2007). We determined the differences in food intake between fed *ad lib*, starved > fed, or starved > fed 30 min (flies that were starved for 23.5 h and then fed for 30 min prior to the experiment; see Materials and methods). As indicated by previous studies, starved > fed flies increase food intake within 30 min compared to their fed *ad lib* controls (Fig. 3B and D) (Itskov et al., 2014; Yapici et al., 2016). Interestingly, we found that food intake is remarkably reduced in starved > fed 30 min flies in comparison to starved > fed flies (Fig. 3B and D). Furthermore, starved > fed