Fly stocks

Flies were raised under a 12 h light:12 h dark (LD) regimen at 25 C on standard corn and yeast media. CantonS from Ralf Stanewsky (University of Münster) were employ for all experiments. All analysed animals were socially naive, unless otherwise stated.

Behaviour

For all experiments 7-8 days old pupae were sorted into glass tubes (70x5x3 mm [length x external diameter x internal diameter]) containing regular food. When animals were 2-3 days old, tubes were loaded in “sleep arenas” and set up in ethoscopes (20 animals per device). In all cases, three days of baseline were recorded before any treatment. All experiments were carried out under LD condition, 50-70% humidity, in incubators set at 25 C. Animals always had *ad libitum* access to regular food. Animals that died during the experiment were excluded from the analysis, except in figure 5.

To evaluate the effect of mating on sleep, a naive male was introduced in the tube of each naive female and allowed to interact for 2 h, from ZT06 to ZT08. After the interaction, males were removed and the activity profile of the females was recorded for another 3.5 days. The short duration of the interaction and the restrictive space of the glass tube reduces the probability of mating. This setup provides the two necessary groups: mated females and females that were courted, but not mated. Effective mating was scored as the presence of larvae in the food four days after the interaction.

The “rotational module” of the ethoscope platform was used to perform the 12 h dynamic sleep deprivation treatments. Different durations of immobility were employed to trigger the rotation of the tube (see figure for details).

To test the effect of long lasting dynamic sleep deprivation on lifespan, the motors of the “optomotor module” were employed, and 20 seconds of immobility used to trigger the tube rotation. At day 7 of the recording, flies were transferred to fresh tube in order to ensure good quality food during the entire experiment. After 9.5 days of dynamic sleep deprivation animals were allowed to recover for 3 days in the ethoscopes at 25 C. After the recovery phase, individual flies were transferred to fresh glass tubes (animals remained individually housed) and moved to 29 C. Mortality was score daily and flies were transferred to fresh tube every 10 days.

Second evaluation of sleep (correlation)

To study the individual consistency of sleep amount over time, behaviour of both males and females was recorded for six days. After the initial recording, individual animals were transferred to fresh tubes. In order to avoid confounding effects related to the location of the tube on sleep amount (e.g. an ethoscope and incubator), the new position of all the tubes was systematically interspersed (see Hurlbert 1984). Namely, low and high sleepers from the same experiment and sex were paired as neighbours in a new arena and their behaviour was recorded for another week.

Behavioural scoring

Immobility was scored by thresholding maximal velocity on ten second epochs as described in Geissmann et al. (2017). Sleep was computed using the five minute rule: immobility bouts longer than 300 s were counted as sleep bouts.

During mechanical sleep deprivation, velocity measurements subsequent to stimuli were masked in order to avoid false positive of fly movement (Geissmann et al. 2017). Specifically, data in the six seconds following the onset of each rotation were not considered for sleep scoring.

Sleep rebound (fig 4D and H) was expressed as the difference between the sleep amount measured during rebound and expected sleep amount. Expected sleep amounts were inferred by a linear regression between reference baseline sleep and sleep during the rebound period, in the relevant control population.

*<Include “Rebound calculation” section from method\_equations.tex>*

Behavioural state (“quiescence”, “micro-movement” and “walking”) was defined for each consecutive minute of behaviour (*B*) according to the following rule:

*<Include “Behavioural state” section from method\_equations.tex>*

The space available inside each experimental tube was variable between individual animals due to the different amount of food and cotton wool. In order to compare flies position with respect to the boundary of their respective experimental environments, their position, in figure 2D, was express relative to the food (position = 0) and the cotton wool (position = 1) edges:

*<Include “Relative position” section from method\_equations.tex>*

Dendrogram

The dendrograms in Fig 3D and S2A are the result of a hierarchical clustering using the Unweighted Pair Group Method with Arithmetic Mean (UPGMA) method (Sokal and Michener, 1958).

During an interval of time, the proportion of time spent by an animal in a behavioural state can be formulated as an empirical discrete probability density function. In this context, the distance between each pair of animal was computed using the average of Bhattacharyya distances (Bhattacharyya 1946) over the entire day:

*<Include “Hierarchical clustering” section from method\_equations.tex>*

Statistics

Unless otherwise stated, the shaded areas around the mean (e.g. Fig. 3A and B) and the error bars (e.g. Fig. 4B-H) are 95% confidence interval computed using basic bootstrap resampling (Efron 1992) with N=1000.

Linear models

The lines in figure 2A and S1B are linear regression and the shaded areas are 95% parametric confidence intervals.

Survival curves

Figure 5F and G are Kaplan-Meier curves. The shaded areas represent a 95% confidence interval.

Software tools

All data analysis was performed in R using the rethomics framework ([https://rethomics.github.io](https://rethomics.github.io/), in preparation). Figures were drawn using ggplot2 and ternary representations in figures 2E and 3C were generated with ggtern.