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**Title:**

Most sleep does not serve a vital function.  
Evidence from *Drosophila melanogaster*

**One sentence summary:**

Sleepless fruit flies live

**Authors:**

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26 **Abstract**

27 Sleep appears to be a universally conserved phenomenon among the animal kingdom but whether  
28 this striking evolutionary conservation underlies a basic vital function is still an open question.  
29 Using novel technologies, we conducted an unprecedentedly detailed high-throughput analysis of  
30 sleep in the fruit fly *Drosophila melanogaster*, coupled with a life-long chronic and specific sleep  
31 restriction. Our results show that some wild-type flies are virtually sleepless in baseline conditions  
32 and that complete, forced sleep restriction is not necessarily a lethal treatment in wild-type  
33 *Drosophila melanogaster*. We also show that circadian drive, and not homeostatic regulation, is the  
34 main contributor to sleep pressure in flies. We propose a three-partite model framework of sleep  
35 function, according to which, total sleep accounts for three components: a vital component, a  
36 useful component, and an accessory component.

37 **Main text:**

38 **Introduction**

39 It is widely speculated that sleep serves a fundamental biological need, an idea derived from three  
40 distinct lines of evidence: (I) sleep is a universally conserved phenomenon across evolution; (II)  
41 chronic sleep restriction is often associated to death; (III) a sleepless animal has never been found  
42 (all reviewed in (1–4)).

43 The first of these three aspects – the striking evolutionary conservation of sleep –  
44 constitutes an important conundrum for scientists, but alone cannot be taken as a proof that sleep  
45 plays a vital function. Circadian rhythms, for instance, are also universally conserved, ultimately  
46 providing a clear evolutionary advantage, but they are not intrinsically vital to the individual given  
47 that animals can survive without a functional internal clock (5).

48 The fundamental question therefore is “can an animal survive without sleep?”. The study of  
49 chronic sleep deprivation could, at least in principle, address this challenge. Unfortunately, the  
50 literature on the chronic effects of sleep restriction is not comprehensive, partly dated and  
51 intrinsically complicated by the many confounding factors that correlate with sleep restriction. To  
52 date, experiments addressing this question have been reported in a handful of species only: dogs  
53 (reviewed in (6)), rats (reviewed in (7)), cockroaches (8), pigeons (9) and fruit flies (10). In four out of  
54 the five tested animal species, sleep deprivation experiments eventually terminated with the  
55 premature death of the animals but the underlying cause of lethality still remains unknown. In rats  
56 and dogs pups, death is associated with a severe systemic syndrome bearing important metabolic  
57 changes and clear signs of suffering, making it difficult to ultimately conclude whether lethality is  
58 caused by the mere removal of sleep or rather by the very invasive and stressful procedures  
59 employed to keep the animals awake (6, 11, 12). In the cockroach *Diploptera punctata*, sleep  
60 deprivation was achieved by continuously startling the animals (8), without however accounting for  
61 exhaustion-induced stress, a known lethal factor for other species of cockroaches (13–15). The  
62 observations in *Drosophila* are limited by the small number of animals used for the only reported  
63 experiment (12 flies in total) and by the methodology employed (human experimenters tapping  
64 their fingers on a tube (8)). In pigeons, chronic sleep deprivation was shown not to be lethal (9). In  
65 conclusion, chronic sleep deprivation experiments appear suggestive but inconclusive, for multiple  
66 reasons.

67 The third line of evidence supporting the hypothesis that sleep serves a fundamental  
68 biological need is perhaps the strongest and concerns the fact that sleepless individuals could never

69 be identified, neither in nature nor through artificial laboratory screenings. We know that some  
70 species – such as elephants (16) or giraffes (17) – have evolved to cope with limited amount of sleep  
71 and several genetic mutations conferring short sleeping phenotypes in flies, rodents, and humans  
72 have been characterised in the past two decades (reviewed in (18)); some animals are also able to  
73 forego sleep for days or weeks in particular ecological conditions (16, 19–21), but the identification  
74 of a constantly sleepless animal can be considered a holy grail of the field.

75         Given that we do not possess a description of sleep at the cell-biological level, in all animals  
76 sleep quantification rely exclusively on *bona fide* macroscopic correlates, either electrophysiological  
77 or behavioural. Therefore, a technological development able to improve the characterisation of  
78 such correlates may provide a more accurate description of sleep, laying the conditions for a more  
79 specific sleep deprivation procedure. To this end, we recently created a system that allows for a  
80 faithful high-throughput analysis and manipulation of *Drosophila* sleep using activity as its  
81 behavioural correlate (ethoscopes (22)). Here, we report two surprising findings that were  
82 uncovered using such system, challenging the notion that sleep is a vital necessity: the discovery of  
83 virtually sleepless flies, and the finding that chronic sleep restriction is largely not lethal in  
84 *Drosophila melanogaster*.

85

## 86 **Results**

### 87 **Virtually sleepless flies are found in a non-mutant population**

88 Prolonged periods of inactivity are an evolutionary conserved, experimentally convenient  
89 behavioural correlate of sleep (23). Absence of movement is therefore routinely used as a proxy to  
90 measure sleep across a wide range of animals, spanning from jellyfish to elephants (16, 24, 25). In  
91 *Drosophila* too, sleep can be estimated by measuring the absence of walking bouts, generally using a  
92 commercially available device to detect whenever an isolated fly crosses the midline of a tube (26).  
93 This system, however, provides only limited spatial resolution which – unsurprisingly – results in  
94 an overestimation of sleep amounts (27). A growing number of laboratories are therefore  
95 transitioning to more accurate systems based on computer assisted video-tracking (27–31). To  
96 further improve our confidence in sleep estimation, we recently introduced a machine-learning  
97 approach that uses supervised learning to detect not only walking activity, but micro-movements  
98 too: *e.g.* in-place movements such as grooming, egg-laying, and feeding (22). How much do flies  
99 really sleep when, beside their walking activity, we measure their micro-movements too? To answer  
100 this question we analysed sleep for four consecutive days in 485 male (Fig. 1A) and 881 female (Fig.

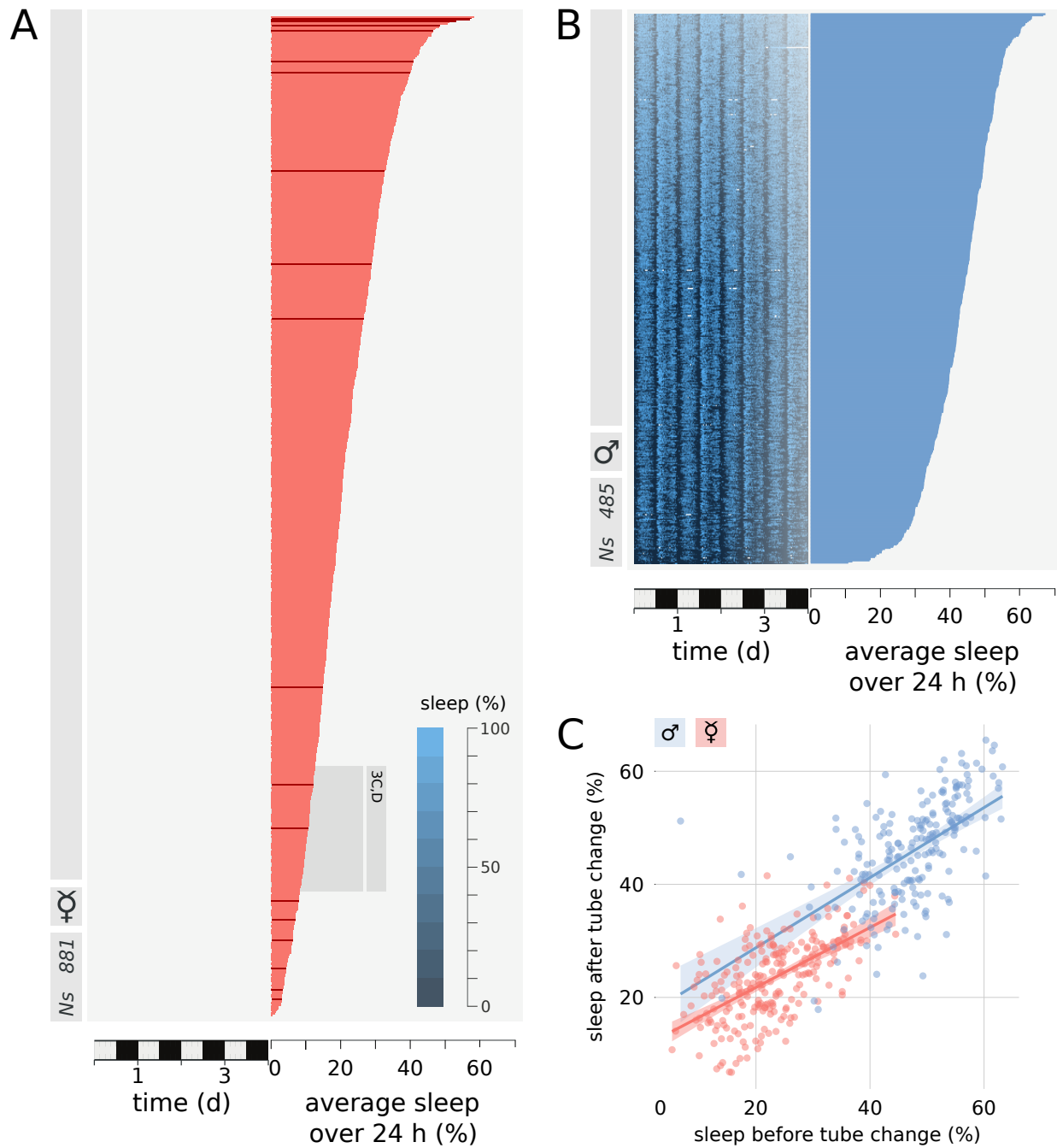
101 1B) socially naive CantonS flies, a commonly used laboratory “wild-type” strain. As expected, in  
102 both males and females, sleep amounts were widely distributed, with male flies sleeping for 618.5,  
103  $CI_{95\%} = [606.7, 630.3]$ , minutes a day and female flies for 299.2,  $CI_{95\%} = [288.8, 309.6]$ , minutes a day  
104 (mean, 95% bootstrap confidence interval; Fig. 1B). Interestingly, the distribution of sleep amount in  
105 females was wider than in males, with a long tail uncovering a previously undescribed fraction of  
106 short-sleeping female flies: 50% of female flies slept less than 20% of their time and 6% slept for less  
107 than 5% of their time (72 minutes a day). At the very end of the curve lied three flies that  
108 spontaneously slept an average of 15, 14, and 4 minutes a day respectively (Fig. 1A and Fig. S1). In  
109 both males and females, sleep amount is an endogenous feature: when flies are transferred into a  
110 novel environment – *i.e.* a fresh tube in a novel ethoscope inside a different incubator – their sleep  
111 amount remains mostly similar to their past sleep (Fig. 1C,  $R^2 = 0.77$ ,  $CI_{95\%} = [0.73, 0.81]$ ).  
112

**Figure 1.** Great variability in sleep amounts in a non mutant population of *Drosophila melanogaster*. (A) Descending sorted distribution of sleep amount in a group of 881 female and (B) in a group of 485 male CantonS flies. The left panel shows sleep amount for each individual over a period of 5 days in bouts of 30 minutes. The right panel indicate the average sleep amount in 24 hours for female (pink in A and the rest of the figures) and male (cyan in B and the rest of the figures) flies. The group of short sleeping flies highlighted in grey is used for cluster analysis in Figure 3C and D. (C) Average sleep amount measured in a tube, predicts sleep amount measured in a different tube. Average of six days for both, with one day in between,  $N_{\text{male}} = 242$  and  $N_{\text{female}} = 242$ .

113

### 114 **Micro-movements explain the short sleeping phenotype**

115 Short sleeping flies have been identified in the past, either through experimental selection (32, 33),  
116 or through selected mutagenesis (34), but flies (and in fact animals) sleeping as little as few minutes  
117 a day where never identified before. To confirm the validity of our results, we reviewed the  
118 positional tracings of all 881 female flies in the dataset (Fig. S1), and acquired and reviewed videos  
119 for 19 flies with representative sleep amounts ranging from 823 to 42 minutes a day, to compare the  
120 tracking record at the single fly level (Fig. S2 – raw videos available at (35)). Manual inspection (Fig.  
121 S1, S2) and quantitative analysis (Fig. 2) confirmed that the activity repertoire oscillates in a  
122 stereotyped, sexually dimorphic, manner (Fig. 2A), with micro-movements being mostly present in  
123 females (Fig. 2B – in females, 623.4,  $CI_{95\%} = [615.3, 631.4]$ , minutes a day had at least one micro-



**Figure 1**

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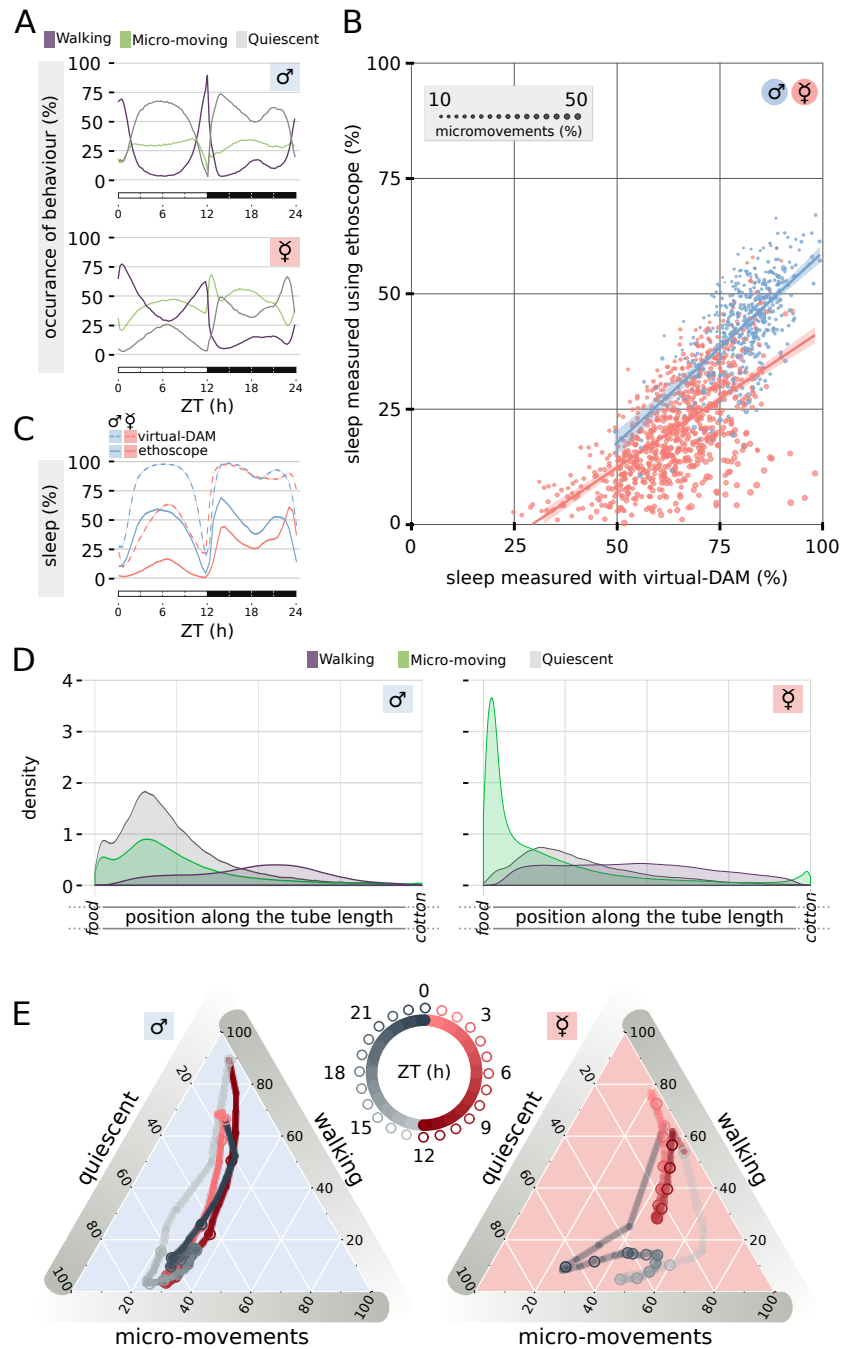
124 movement episode, whilst in males 411.4,  $CI_{95\%} = [404.2, 418.8]$ , minutes did; mean, 95% bootstrap  
125 confidence interval). As expected, micro-movements (Fig. 2B) and movements that do not span the  
126 entire tube length (Fig. S1) are responsible for the quantitative difference in sleep analysis between  
127 recording platforms (Fig. 2C). Importantly, female micro-movements and quiescence are spatially  
128 (Fig. 2D) and, to a certain extent, temporally (Fig. 2A) exclusive: 37.3%,  $CI_{95\%} = [36.9, 37.7]$ , of the  
129 micro-movements happen at night (ZT12-ZT21), of which 51.3%,  $CI_{95\%} = [50.3, 52.2]$ , within 4 mm from  
130 the food (Fig. 2A and 2D respectively, green). In short, expanding on previously reported findings  
131 (36, 37), micro-movements in females are concentrated to those times of the day when flies are  
132 known to increase feeding activity (*i.e.* during mid-day and in the early phase of the night), mostly  
133 located by the food and away from the preferred site for quiescence (Movie S1 and Fig. 2),  
134 suggesting that the micro-movements observed in female flies are not a sleeping-related behaviour  
135 but a feeding-related behaviour.  
136

**Figure 2.** Micro-movements account for the newly described short sleeping phenotype. (A) Average occurrence of behaviour over the 24 h period in male (upper panel) and female (lower panel) Canton S flies. (B) Sleep amount for each individual male (cyan) and female (pink) fly plotted as computed with ethoscopes (y axis) and with virtual DAM analysis (29)(x axis). The size of each dots represents the average amount of micro-movements observed over the 24 hour period. (C) Average sleep amount over the 24 h period in male and female flies, plotted as computed with ethoscopes (continuous lines) or virtual DAM analysis (dashed lines). (D) Average positional distribution of behaviours for male (left panel) and female (right panel) flies over the 24 hour period, broken into the three behavioural states identified by ethoscopes. (E) Four dimensional representation of behavioural transitions over the 24 h period. Grey shades indicate the dark period (ZT 12-24), while red shades indicate the light period (ZT0-12). Same data set shown in Figure 1A,B.

137

### 138 **Qualitatively different types of short sleeping females**

139 High-throughput ethoscope analysis allowed us to identify wild-type female flies that sleep as little  
140 as few minutes a day (Fig. 1B, Fig. S1). Could this be a peculiarity of some virgin flies, hence an  
141 ethological laboratory artefact? In *Drosophila*, mating status is known to be acting as a major  
142 behavioural switch (38) that modifies, among other behaviours, the animals dietary preference (36,  
143 39) and their preference for feeding time (40). However, reaching sexual maturity only few hours  
144 after ecdysis, virgin female flies are likely to be a rare occurrence in the wild (41, 42). To test how



**Figure 2**  
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145 sleep changes with mating status, we recorded sleep in flies before and after a successful (green), or  
146 unsuccessful (grey), mating event (Fig. 3A). In mated females, sleep dramatically decreased for at  
147 least three consecutive days (Fig. 3A – in the first full day after mating, lowering from 383.2,  
148  $CI_{95\%} = [363.4, 404.9]$ , to 175.3,  $CI_{95\%} = [153.0, 200.2]$  minutes a day), correlating with a major change of  
149 positional preference of the animals towards the food (Fig. 3B). Such change in positional  
150 preference (Fig. 3B) and the strong increase in micro-movements (Fig. 3C) are likely to represent an  
151 increase in food intake and egg-laying activity and may explain why such a strong decline in sleep  
152 amount was never identified using different tools (27, 43, 44). Interestingly, four-dimensional  
153 behavioural fingerprinting showed that the short sleep phenotype observed upon mating is  
154 qualitatively different from the one observed as natural variation in the CantonS population (Fig.  
155 3C, D and Fig. S2).  
156

**Figure 3.** Mating reduces sleep amount. (A) Sleep profile of all the female flies used in the mating experiment: Green: flies that underwent successful mating event (N = 86); Grey: flies that met a male but did not engage in copulation (N = 152). The blue vertical shade indicates the timing of the mating event. (B) Average position along the tube of the same flies shown in A in 30 minutes bins. (C) Four dimensional representation of behavioural transitions over the 24 h period for non-mated flies (grey background), mated flies (green background) and for naturally short sleeping unmated flies (pink background – same dataset highlighted in grey in Figure 1A). (D) Hierarchical clustering based on pairwise distance, in the time-behaviour domain, of the same three cohorts shown in C.

157

### 158 **Prolonged sleep deprivation is largely non lethal**

159 The experiments described so far unveiled that a fraction of female flies necessitate little sleep,  
160 with some being almost completely sleepless. Is sleeplessness a peculiarity of a few special  
161 individuals, or can any fruit fly cope with little or no sleep? To answer this question, we conducted  
162 a life-long sleep deprivation experiment using a closed-loop sleep deprivation device able to interact  
163 with single animals by triggering a tube rotation after a predefined period of immobility (22), a  
164 system created to minimize the extent of disturbance and conceptually inspired by the disc-over-  
165 water apparatus developed by the Rechtschaffen laboratory (7) in which a rat receives a waking  
166 physical challenge only when it is factually asleep, but is left undisturbed otherwise. In our setup,  
167 flies were housed in individual tubes and each tube experienced a 1 second rotation at the  
168 approximate speed of 300 rpm whenever the animal housed inside had shown 20 s of continuous

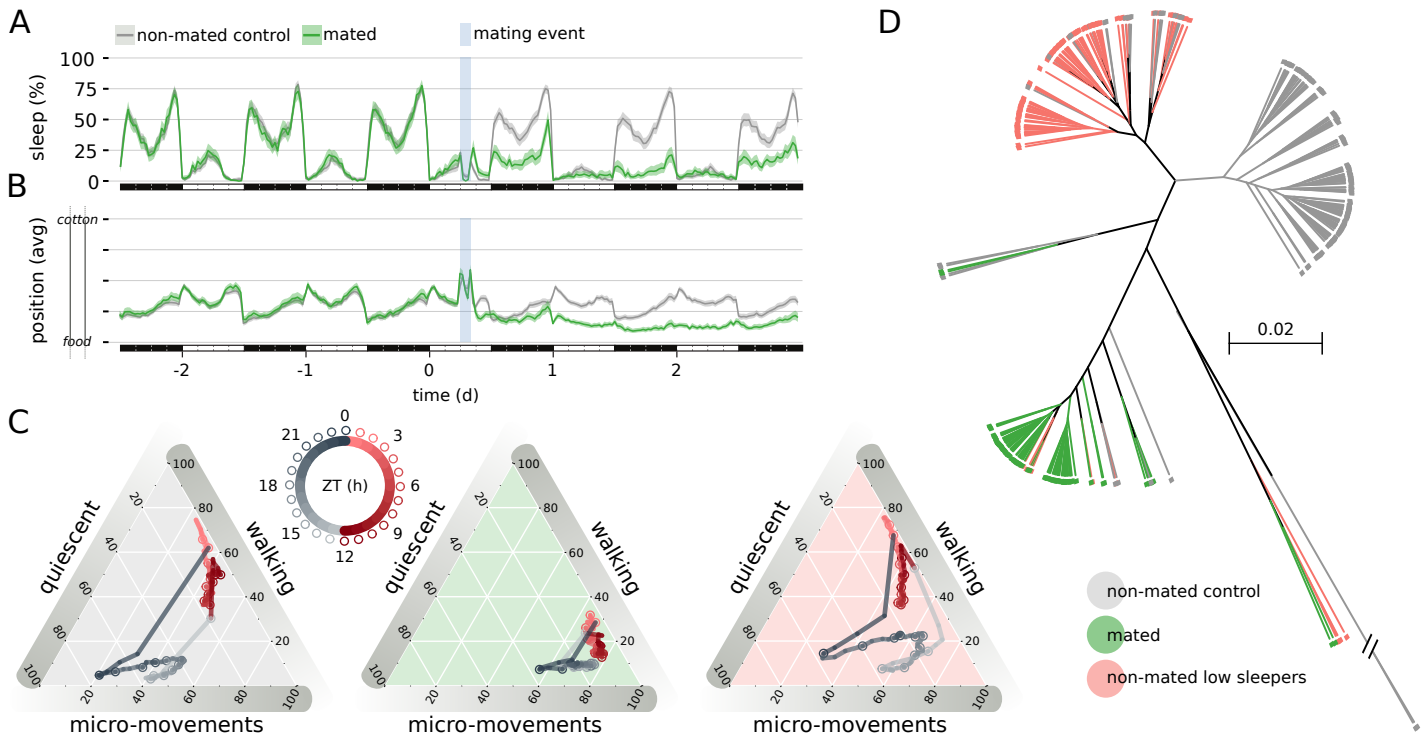


Figure 3

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168 immobility (22). The treatment led to a highly efficient sleep deprivation with flies losing, on  
169 average, 95.6%,  $CI_{95\%} = [93.5, 98.2]$ , of their sleep (Fig. 4A) and yet, surprisingly, we could not detect  
170 any major effect on survival (Fig. 4B, C). In particular, sleep deprived male flies lived as long as the  
171 control group (with a median of 41.5,  $CI_{95\%} = [38.0, 44.0]$ , days against 46.0,  $CI_{95\%} = [41.0, 48.5]$ , days for  
172 the controls) and a minor effect was only evident in female flies, with a reduction of median  
173 lifespan of 3.5 days (37.5,  $CI_{95\%} = [33.0, 38.5]$ , 41.0,  $CI_{95\%} = [38.5, 44.0]$ ). Forced sleep restriction is largely  
174 not lethal in flies when performed in a controlled, specific, manner.

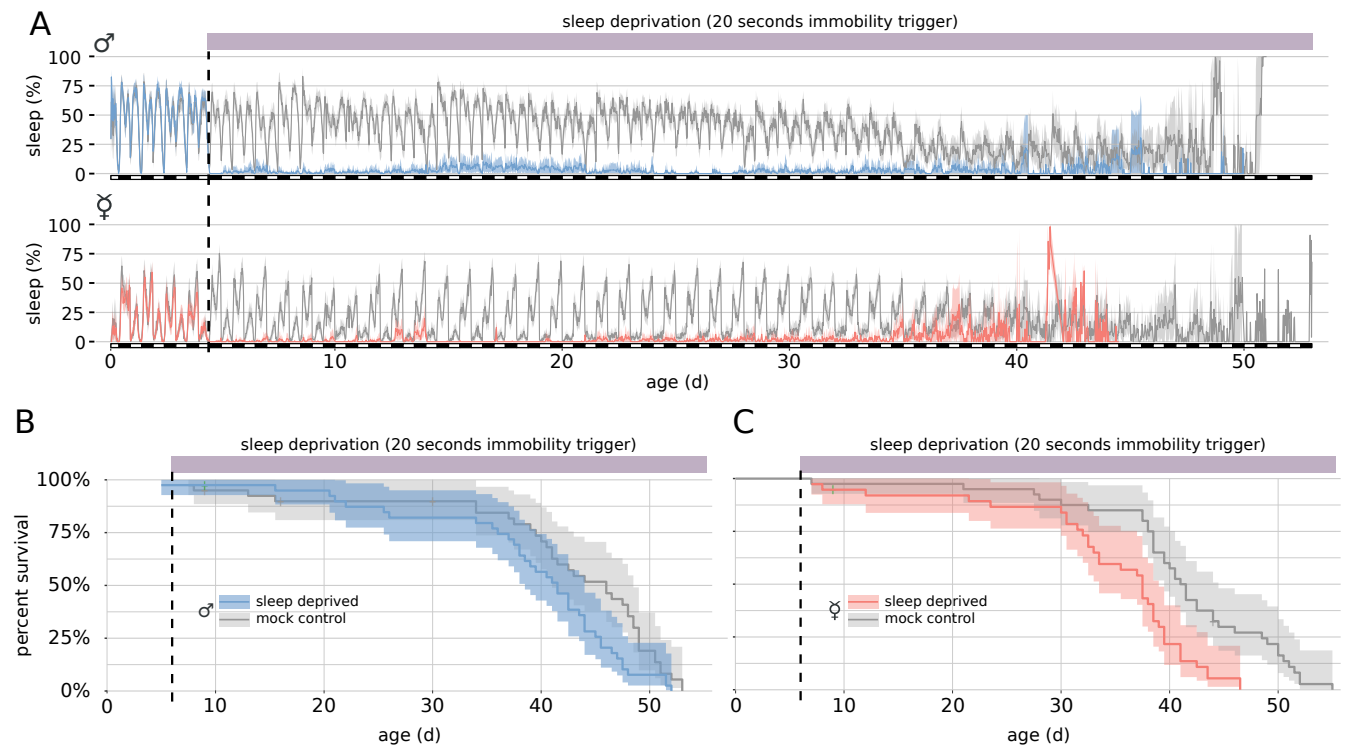
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**Figure 4.** Chronic mechanical sleep deprivation is largely not lethal in *Drosophila melanogaster*. (A) Life-long sleep restriction in male (upper panel) or female (lower panel) CantonS flies subjected to mechanical sleep deprivation triggered by a 20 s inactivity bout. (B) Survival curve for male (cyan in B) or female (pink in C) sleep deprived flies and their sex-matched undisturbed control (grey in both B and C). Sleep measurements became noisier as the number of flies decreases.  $N = [38, 40]$  for all four groups.

176

#### 177 **Sleep rebound after sleep deprivation only partly correlate with sleep loss**

178 If most (all?) sleep does not serve a direct and immediate vital function, do we need to rethink the  
179 current prevailing concept of sleep homeostasis? Is sleep rebound a way to make up for a loss of an  
180 otherwise impaired biological process, or is it instead merely a “punishment” phenomenon, evolved  
181 to guarantee that a constant, largely species-specific amount of sleep is met? To explore this new  
182 dichotomy, we analysed how different treatments of sleep deprivation would affect sleep rebound.  
183 To start, we conducted an acute sleep deprivation experiment on a total of 818 male (Fig. 5A-D) and  
184 992 female (Fig. 5E-H) CantonS flies, with a comprehensive range of immobility triggers, spanning  
185 from 20 to 1000 seconds, to deprive flies of sleep episodes of specific length. As expected, the total  
186 amount of sleep lost during the 12 h of deprivation positively correlated with the length of the  
187 immobility trigger adopted (Fig. 4B,F) whilst the number of stimuli delivered was inversely  
188 correlated (Fig. 4C,G). Interestingly, in all cases could we observe a statistical significant sleep  
189 rebound in the first 3 hours following the sleep deprivation, also when the sleep loss was not  
190 statistically different than control (Fig. 4F, 840 s and 1000 s inactivity triggers). In particular,  
191 depriving female flies of only the longest sleep episodes ( $\geq 1000$  s) still led to a significant sleep  
192 rebound the subsequent morning, even though flies experienced, on average, only 5.8,  $CI_{95\%} = [4.8,$   
193 6.8], of tube rotations per night.



**Figure 4**  
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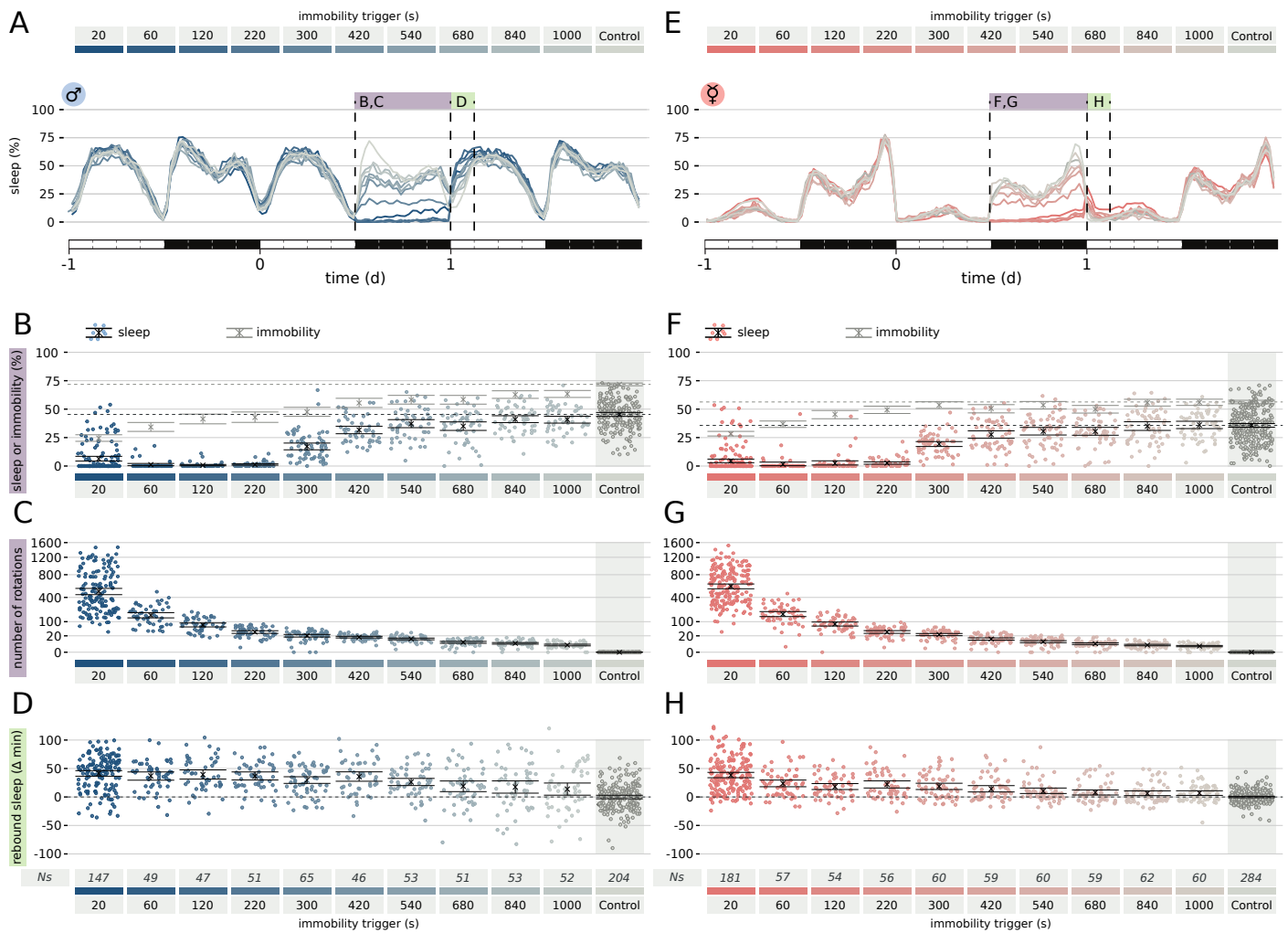
**Figure 5.** Sleep rebound is not linearly proportional to sleep loss. (A, E) Sleep profile for the entire data set shown: 818 male (A-D) and 912 female (E-H) CantonS flies. (B, F) Sleep (cyan and pink dots and black lines) or immobility (grey lines) for the entire data set spanning 10 different immobility interval triggers (20 s to 1000 s). Control flies were never actively stimulated but laid adjacently to the experimental flies. (C, G) Number of tubes rotations triggered by immobility bouts. (D, H) Amount of rebound sleep in the ZT0-3 interval following the sleep deprivation for the entire data set.

194

195 The increase in sleep pressure driving rebound after sleep deprivation is not linearly correlated  
196 with the amount of sleep lost over the length of one night, but how do flies react to prolonged sleep  
197 restriction spanning multiple days? To answer this question, we conducted a “Randy Gardner”-like  
198 experiment (45), in which we subjected flies to 228 h of uninterrupted sleep deprivation, using a 20 s  
199 immobility trigger as waking event. The experiment was conducted both in males and females flies,  
200 using undisturbed control individuals in adjacent tubes, for a total of 377 animals (Fig. 5). Even after  
201 almost 10 d of chronic sleep deprivation, male flies manifested a sleep rebound that was not  
202 dissimilar from the rebound observed after one night of acute sleep restriction (visually compare  
203 Fig. 6C to Fig. 5A). Intriguingly, whilst in male flies rebound sleep was again limited to the first three  
204 hours of rebound day, in female flies the observed sleep rebound was quantitatively modest but  
205 appeared to be protracted in time for the subsequent three days, at least (Fig. 6B and 6D). Because  
206 the tube rotations were triggered by immobility, we could use the number of rotations (Fig. 6F,  
207 dashed lines) and the distance walked (Fig. 6E, continuous lines) as proxy of endogenous sleep  
208 pressure in control (grey) or sleep deprived (pink and blue) flies (Fig. 6F).

209

**Figure 6.** Sleep pressure is largely under control of the circadian rhythm. (A, B) Sleep profile for male (A, cyan) and female (B, pink) CantonS flies during the length of the experiment compared with their sex-matched undisturbed controls (grey in both). Day 0 signs the beginning of the chronic sleep deprivation procedure, lasting 228 hours (indicated by a purple shade on top). The green shade indicates the rebound day blown up in C and D. (C, D) Magnification of the sleep deprivation to rebound transition. (C', D') Quantification of sleep amount during ZT0-3 of rebound day. (E) Activity of flies shown as distance walked (continuous lines: grey, control; cyan, males; pink, females) or, by proxy, as number of rotations over the average 24 hours period (dashed lines: cyan, males; pink, females). (F) Actual average number of tubes rotations over the length of the



**Figure 5**  
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sleep deprivation experiment (dashed lines) or seasonal trend (continuous lines – see supplementary methods for details). N = [93, 95] for all four groups.

210

211 In both male and female flies, the main changes in sleep pressure were cycling in a circadian  
212 fashion, with the clock-regulated bouts of walking activity still showing no sign of subsidence,  
213 despite the long sleep deprivation (Fig. 6F). In other words: when the circadian clock commands  
214 activity, the flies are active also after days and days of cumulating sleep pressure. In fact, seasonal  
215 decomposition of rotations over the 9.5 d of sleep deprivation confirmed that only a small amount  
216 of the variance in sleep pressure is explained by the long range trend in sleep deprivation (21% in  
217 males and 11% in females, Fig. 5F), whilst the main contributor of sleep pressure is indeed circadian  
218 periodicity (69% in males and 61% in females, Fig. 5F continuous lines). These data, taken together,  
219 clearly indicate that the main stimulus to rest in flies is driven by the circadian clock.

220

## 221 **Discussion**

222 The idea that sleep fulfils a vital biological need – we initially argued – relies on one fundamental  
223 question: can we find an animal able to survive without sleep? According to the data presented  
224 here, the answer could be “yes”. In wild type *Drosophila melanogaster* the need for sleep is not a vital  
225 necessity and lack of sleep – either endogenously driven (Fig. 1) or artificially imposed (Fig. 4 and 6)  
226 – is compatible with life. The utmost conceptual importance of these findings commands caution,  
227 and caveats must be critically examined. Most importantly, we cannot rule out that, in our  
228 experiments, flies still experience enough sleep to satisfy an hypothetical vital need. In other words,  
229 prolonged or consolidated sleep is not a vital necessity but intervals of sleep that last only few  
230 seconds (20 s in most experiments here presented) may be sufficient to satisfy whatever basic  
231 biological need sleep may serve. Behavioural correlates of sleep have been described in virtually  
232 every animal that has been studied so far, connecting species as different as jellyfishes and humans  
233 (25, 46), and have demonstrated that sleep amounts vary dramatically across the animal kingdom.  
234 For instance, elephants sleep as little as 3 hours a day (16), Tinaja cavefish as little as 2 hours a day  
235 (47), whereas little brown bats sleep, on average, 20 hours a day (46). No existing model of sleep  
236 function can account for this variability. One intriguing possibility, which we propose to the reader  
237 here, is that sleep should not be seen as a monolithic phenomenon but rather as the mixture of  
238 three components: a *vital* component, a *useful* component, and an *accessory* component. The  
239 accessory component is conceptually identical to what has been previously coined “adaptive

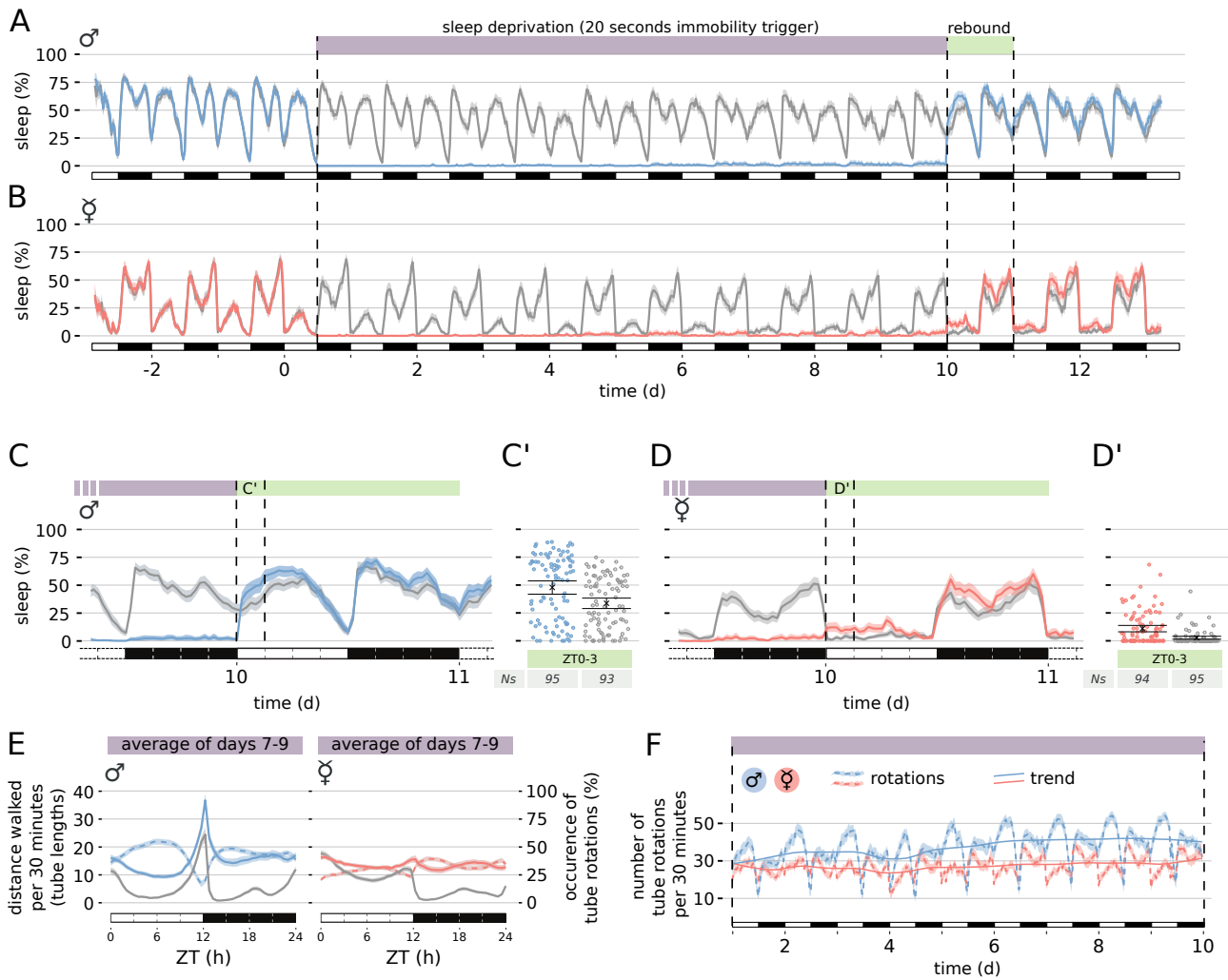


Figure 6

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250 inactivity” (2) or “trivial function of sleep” (3) and would postulate that at least a fraction of sleep  
251 would serve no core biological function other than circadianly syncing periods of wakefulness in  
252 the most ecologically appropriate manner, for instance keeping animals out of danger or  
253 restricting their activity to gain safety and rest. Can we accept that a good fraction of those 20  
254 hours of sleep a brown bat requires is driven by the evolutionary adaption of staying “out of  
255 trouble”? And, if this is intuitively easy to accept for bats, why should it not be universally true?  
256 Other vital needs, such as feeding, follow a similar three-partite subdivision, with a given amount  
257 of calories and nutrients being vitally needed, some useful, and some merely accessory – and even  
258 detrimental. In our experiments, we may have removed the last two components (accessory and  
259 useful) but left enough sleep to satisfy a yet mysterious vital need, even if in the form of short bouts  
260 lasting few seconds each. We also uncovered an interesting sexual dimorphism in terms of  
261 undisturbed sleep need and in terms of response to sleep deprivation: whilst female flies are able to  
262 cope with much less sleep in baseline conditions, they are more sensitive to sleep deprivation, with  
263 an extended rebound upon long sleep restriction (Fig. 6B and D) and a moderate but significant  
264 effect on lethality upon life-long sleep deprivation (Fig. 4). This sexual dichotomy may be  
265 instrumental in the future to dissect the difference between the three-partite components.

266 At first sight, the results presented here appear to be clashing with some of the existing  
267 knowledge. In our view, they command, instead, for a thorough review of existing sleep deprivation  
268 literature. The experiments of chronic sleep deprivation performed in dogs pups at the end of 1800s  
269 are universally considered too primitive to be trustworthy and too unethically stressful to be  
270 reproducible in modern times (6). The early *Drosophila* experiments were too preliminary to depict  
271 a whole picture, marred by a limited number of animals (12 individuals) and by the adoption of a  
272 procedure that is not easily reproducible (human experimenters finger tapping on the tubes) (10).  
273 Other lines of research have also shown no correlation between sleep loss and survival in flies: loss  
274 of the *insomniac* (48) or *fumin* (34) genes leads to strong sleep restriction that is still compatible with  
275 life. Likewise, artificially selected short-sleeping fruit flies have unaltered longevity (32). With flies  
276 joining pigeons in the list of animals surviving chronic sleep deprivation, the only solid evidence in  
277 favour of lethality upon sleep deprivation lays with the chronic sleep deprivation in rats using the  
278 disc-over-water system. Those experiments, however, were not free of confounding factors and one  
279 cannot exclude a stress or metabolic component given that animals were thrown into water several  
280 hundreds times a day (12). In humans, for obvious ethical reasons, we have no experimental  
281 evidence that prolonged sleep deprivation is incompatible with life. A human prion diseases, fatal

282 familial insomnia (FFI), is sometimes brought as evidence of a vital function of sleep, yet bearing  
283 too many confounding factors, considering the devastating nature of the pathology (49).  
284 Importantly, transmitted (50) and transgenic mouse models (51) of FFI reproduce clear signs of  
285 neurodegeneration and premature death, but not sleeplessness suggesting that, in humans, the  
286 insomnia is a symptom of the disease but not necessarily the cause of death (52). In conclusion, we  
287 believe our results clearly show that the time is ripe for the field to revisit the dogmatic believe that  
288 sleep serves a unique, evolutionary conserved, function.

289

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297 competing interests. **Data and material availability:** all data, raw and processed, is available in the  
298 manuscript or the supplementary materials.

299

#### 300 **Supplementary Materials:**

301 Materials and Methods

302 Figure S1

303 Figure S2

304 Movie S1

305 **Figure S1.** Representative tracings of the behavioural activity over the course of 48 h as recorded in  
306 real-time by the ethoscopes for all 818 female flies shown in Figure 1A. The continuous black line  
307 plots the position of the flies along the tube (y axis) over time (x axis). The transversal dashed line  
308 represents the position of a virtual infrared beam (29). The colour on the background highlights the  
309 concomitant behavioural classification with a resolution of 1 minute (grey: quiescent, green: micro-  
310 moving; blue: walking).

311

312 **Figure S2.** Sorted hierarchical cluster analysis based on pairwise distance, as supplement to Figure  
313 3. (A) Average daily sleep amount as % of the day for each female fly in the dataset. Same colour  
314 code as Figure 3. (B) Hierarchical clustering dendrogram based on pairwise distance. (C) Visual  
315 representation of the average occurrence of the three behavioural features in each single animal  
316 across the 24 hour period.

317

318 **Movie S1.** Visual representation of the distribution of behaviour features across the 24 h in the  
319 dataset shown in Figures 1A,B and 2. Each frame in the movie bins data with a 15 minutes  
320 resolution: red: female flies; blue: male flies. In the leftmost panels, each dot is an individual animal  
321 plotted in their behavioural space at that time point. The right most panel shows the 24 h  
322 distribution of the following four features: fraction of quiescent, micro-moving, walking animals  
323 (top three) and average position along the tube longitudinal length (bottom).

324

## 325 **References and notes**

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