A potassium channel β-subunit couples mitochondrial electron transport to sleep

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The essential but enigmatic functions of sleep1,2 must be reflected in molecular changes sensed by the brain's sleep-control systems. In the fruitfly Drosophila, about two dozen sleep-inducing neurons3 with projections to the dorsal fan-shaped body (dFB) adjust their electrical output to sleep need4, via the antagonistic regulation of two potassium conductances: the leak channel Sandman imposes silence during waking, whereas increased A-type currents through Shaker support tonic firing during sleep3. Here we show that oxidative byproducts of mitochondrial electron transport6-7 regulate the activity of dFB neurons through a nicotinamide adenine dinucleotide phosphate (NADPH) cofactor bound to the oxidoreductase domain8-9 of Shaker's Kβ3 subunit, Hyperkinetic10,11. Sleep loss elevates mitochondrial reactive oxygen species in dFB neurons, which register this rise by converting Hyperkinetic to the NADP⁺-bound form. The oxidation of the cofactor slows the inactivation of the A-type current and boosts the frequency of action potentials, thereby promoting sleep. Energy metabolism, oxidative stress, and sleep—three processes implicated independently in lifespan, aging, and degenerative disease8-14—are thus mechanistically connected. Kβ3 substrates8,15,16 or inhibitors that alter the ratio of bound NADPH to NADP⁺ (and hence the record of sleep debt or waking time) represent prototypes of potential sleep-regulatory drugs.

Like sleep-active cells of the mammalian hypothalamus17, dFB neurons tend to be electrically active during sleep. To cause awakenings, dopamine, acting directly on these cells2, attenuates the voltage-gated A-type current18 (Iₐ) carried by Shaker and augments a voltage-independent leak current through the two-pore domain channel Sandman5. Sandman translocates from an intracellular storage pool to the plasma membrane, where it opens a potassium shunt that short-circuits the spike generator and switches the sleep-inducing neurons OFF5. The reverse switch, of dFB neurons to their electrically active state, is at the core of the organism's response to sleep loss5. We therefore able to frame the question of sleep's biological role as a mechanistically well-defined problem: which signals or processes switch dFB neurons ON? Knowing the leading parts played in the excitability switch by Shaker and Sandman focuses the search for answers on two events: the upregulation of the Shaker current, and the internalization of Sandman. Our spotlight here is on Shaker.

Shaker coassembles with a β-subunit called Hyperkinetic10 (Hk) into a voltage-gated potassium channel with fourfold rotational symmetry4. The α-terminal domains of four Shaker α-subunits form a hanging platform, suspended below the voltage sensors of the channel, to which a cytoplasmic Hyperkinetic tetramer docks. Hyperkinetic and other Kβ3 subunits are related in sequence10,19 and structure6,9 to aldo-keto reductases, complete with a catalytic tyrosinate anion, charge-relay system, and NADPH cofactor in their active sites. Oxidation or reduction of the stably bound nicotinamide by small-molecule substrates8,15,16 can alter the kinetics of voltage-dependent activation and/or inactivation of the channels10,15,20. The functional purpose of coupling a cell's excitability to its metabolism, however, remains obscure8,9.

Mutations in either Shaker or Hyperkinetic cause insomnia11,13. Unsurprisingly, given the importance of Iₐ for sustaining the sleep-promoting activity of these cells3, dFB neurons are a major sleep-relevant site of action for both channel subunits: the depletion of either gene product from these cells alone, using R23E10-GAL4-restricted4 RNA interference (RNAi), reproduces the sleep disruptions caused by the genomic mutations5. To complement these demonstrations of necessity with a test of sufficiency, we restored Hyperkinetic expression exclusively in the dFB of otherwise homozygous mutant flies. Sleep returned to wild-type levels, but only if the active site of Hyperkinetic was intact (Fig. 1a, b): a putative rescue transgene encoding a variant17 with a point mutation (K289M) that abolishes the protein's oxidoreductase activity15,20 but leaves its expression15,20,21 and the amplitude of Iₐ unaltered (see below) proved ineffective. This finding has three implications. First, it suggests that the sleep-regulatory role of Hyperkinetic is tied to its ability to sense changes in cellular redox state, which are therefore expected to accompany changes in sleep pressure. Second, it predicts that perturbing the redox chemistry of dFB neurons should have consequences for sleep. And third, it identifies a biophysical mechanism for coupling redox chemistry and sleep. Because redox reactions, oxygen use, and ATP synthesis are linked at the level of the flow of reducing equivalents through the mitochondrial electron transport chain, dFB neurons may monitor redox processes as a gauge of energy metabolism.

To examine the first of these implications, we compared the redox histories of flies that had been mechanically sleep-deprived with those of rested controls (Fig. 1c, d). The metabolic machinery of the inner mitochondrial membrane is the principal cellular source of oxidants, especially under conditions of ample NADH supply, large proton-motive force, and low ATP demand, when electrons stall in the transport chain and transfer directly to oxygen6,7, producing superoxide (O₂⁻) that is subsequently dismutated to H₂O₂ (Fig. 2a). The chief conduits for electron leakage7 are a fully reduced ubiquinone pool and the resulting tailback of electrons onto complex I. Although some reactive oxygen species (ROS) generated in mitochondria could conceivably reach the active site of Hyperkinetic by diffusion, a more plausible scenario is that O₂⁻ and H₂O₂ react locally and release a longer-lived carbonyl substrate6, whose reduction by Hyperkinetic then causes the oxidation of NADPH. Lipid peroxidation products, such as the aldehyde 4-oxo-2-nonenal, serve as established hydride acceptors in Kβ3 subunits15,16 and may represent the ill-defined electron densities8 that overlie their hydrophobic active sites.

For a cumulative estimate of ROS production, we labelled the mitochondria of dFB neurons with a matrix-targeted fluorescent protein (MitoTimer) whose green-emitting chromophore converts irreversibly to red when oxidized22. We then deprived age-matched flies of variable amounts of sleep and determined the fluorescence emission ratio by two-photon microscopy. Mitochondrial ROS production rose with the size of the imposed sleep deficit: a night of sleep deprivation red-shifted MitoTimer's fluorescence relative to rested controls, but applying the same sleep deprivation protocol during the day, when flies are mostly awake, or adding a day to a night of sleep disruption
To strengthen this connection, we quantified sleep after three further dFB-neuron-specific interventions: manipulation of mitochondrial electron transport; chronic interference with antioxidant enzymes; and acute optogenetic induction of singlet oxygen (\(\cdot{O}_2\)) formation.

We first installed an electron overflow pathway in the inner mitochondrial membrane of dFB neurons by expressing the alternative oxidase AOX of Ciona intestinalis\(^{23,24}\). Like complex III, AOX injects ROS when the cytochrome branch of the transport chain is saturated or the availability of ADP is low\(^6,7,23\). The introduction of AOX into the mitochondria of dFB neurons, which normally lack the capacity for alternative respiration, decreased daily sleep by nearly 7 h (Fig. 2c): clamping mitochondrial ROS production eased the pressure to sleep.

In animals without bifurcated electron transport chains, superoxide dismutases (SODs) and catalase, which acts as a sink for SOD-generated \(\cdot{H}_2{O}_2\) and thereby pulls the dismutation reaction forward, form the first line of anti-oxidant defences\(^{6,12}\) (Fig. 2a). Shoring up these defences by overexpressing SOD1 or catalase in dFB neurons reduced sleep (Fig. 2d, e), while breaching them with the help of a mutant enzyme (SOD1(A4V)), whose peroxidase activity is enhanced\(^{25,26}\), had the converse effect; it increased sleep (Fig. 2d) without inhibiting waking locomotion (Extended Data Fig. 1a) or arousability (Extended Data Fig. 1b). The crucial link between redox chemistry and sleep was the Shaker–Hyperkinetic complex: the RNAi-mediated depletion of either channel subunit from dFB neurons not only reduced the sleep-promoting effect of SOD1(A4V) but in addition reduced sleep below wild-type levels (Fig. 2d). By contrast, interference with the expression of Shal (Shaker cognate 1), a Kv channel without a sleep-regulatory function in dFB neurons\(^5\), proved innocuous (Fig. 2d).

Analogous SOD1 manipulations in cryptochrome- or PDF-positive clock neurons or Kenyon cells (all of which have demonstrated roles in sleep control\(^{11,12}\) or in olfactory projection neurons (for which no such role has been reported) failed to influence sleep (Extended Data Fig. 2a–d). dFB neurons thus appear to be unique, at least among this comparison group, in their ability to transduce oxidative stress into sleep.

As a third test of the redox control of sleep, we anchored miniSOG, an engineered flavoprotein\(^{16}\) that photogenerates \(\cdot{O}_2\), via a myristoyl group at the cytoplasmic face of the plasma membrane\(^{22}\) of dFB neurons (Fig. 3a). If the light-driven release of \(\cdot{O}_2\) near Hyperkinetic causes the oxidation of bound NADPH, either directly or via local lipid peroxidation, it should be possible to bypass the chain of events that couples this final transduction step to mitochondrial respiration and induce sleep acutely. Indeed, during a 30-min observation interval that began with a 9-min exposure to blue light, flies expressing miniSOG in dFB neurons fell quiescent in greater proportions, and for longer, than control flies did (Fig. 3b, c). Epochs of quiescence outlasted the illumination period by about 1 h (Fig. 3d), could be blocked by the removal of Hyperkinetic but not of Shal (Fig. 3b, c), and were not due to the suppression of waking movements (Extended Data Fig. 1c) or a confounding influence of cryptochrome photoreceptors\(^3\) (Extended Data Fig. 3).

Whole-cell recordings from dFB neurons, before and after miniSOG-mediated photo-oxidation under sleep-inducing conditions, revealed some of the well-documented biophysical changes that underpin the wake–sleep switch\(^4,5\): the neurons’ action potential responses to depolarization current became more vigorous (Fig. 4a–c); their membrane time constants lengthened (Fig. 4b); the interspike interval contracted (Fig. 4a, c); and fast \(I_h\) inactivation slowed (Fig. 4d, e, Extended Data Table 1). Because A-type channels in the conducting membrane constitute the repolarizing force that returns the membrane potential to its resting level after a spike, changes in their inactivation kinetics influence firing rates\(^18\). Both are regulated by Kv\(\beta\) subunits,
The coherent picture that emerges from these within-cell analyses was mirrored in between-cell comparisons of neurons with chronically altered redox-sensing or redox-buffering capacity: the homozygous K_v mutants carrying catalytically active or dead rescue constructs that were our point of departure (Fig. 4f–j), or cells containing pro-oxidant\textsuperscript{15,20} SOD1(A4V) or anti-oxidant\textsuperscript{23} AOX (Fig. 4k–o). dFB neurons equipped with a functional K_v subunit expressed slowly inactivating A-type currents (Fig. 4i, j) that enabled high-frequency action potential trains (Fig. 4f, h). In flies forced to make do with the Hk(K289M) mutant (15,20,21), which cannot convert NADPH to NADP\textsuperscript{+}, dFB neurons exhibited fast-inactivating I_K (Fig. 4i, j), long interspike intervals (Fig. 4f, h), and shallow current–spike frequency functions (Fig. 4f, h) that can account for the insomnia of these animals (Fig. 1a, b). Profound shifts in the NADP\textsuperscript{+}:NADPH ratio of Hyperkinetic in opposite directions must also underlie the divergent interspike interval distributions (Fig. 4g, m), current–spike frequency functions (Fig. 4k, m), and A-type inactivation kinetics of dFB neurons expressing SOD1(A4V) or AOX (Fig. 4n, o), which parallel large and opposite changes in daily sleep (Fig. 2c, d).

Because K_v subunits have extremely low cofactor exchange rates that limit the number of enzymatic conversions\textsuperscript{15,20}, perhaps to a single hydride transfer\textsuperscript{5}, even a fleeting exposure to an oxidizing substrate will form a lasting biochemical memory. The Shaker–Hyperkinetic complex therefore unites three discrete functions in a single device. Its redox sensitivity allows it to monitor a key process relevant to sleep—the generation of oxidative byproducts of mitochondrial electron transport. Its catalytic inefficiency allows the protein to compute and store the time integral of the resulting oxidative burden, as would be required if the purpose of sleep were to protect against oxidative stress\textsuperscript{13}. And its ability to set the spike frequency via conformational coupling to the channel’s inactivation gate allows it to titrate the commensurate corrective action.
Before dFB neurons can discharge the accumulated sleep pressure, however, the inhibitory clamp imposed by Sandman must be released. This almost certainly involves the regulated endocytosis of the channel\(^2\). We suspect that the movements of Sandman between intracellular storage vesicles and the cell surface are essential for the conversion of continuously varying sleep pressure into discrete sleep–wake states. Sharp transitions, not slow drift through twilight states, are of course continuously varying sleep pressure into discrete sleep–wake states.

Online content
Any methods, additional references, Nature Research reporting summaries, source data, statements of data availability and associated accession codes are available at https://doi.org/10.1038/s41586-019-1034-5.

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Author contributions G.M., S.M.S. and A.K. designed the study and analysed the data. A.K. performed electrophysiological recordings and carried out imaging experiments, molecular manipulations, and behavioural analyses with S.M.S. C.B.T. developed instrumentation and code. G.M. directed the research and wrote the paper.

Competing interests A patent application has been filed by G.M., A.K., S.M.S. and Oxford University Innovation Ltd. on the basis of work described in this study.

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Drosophila strains and culture. Fly stocks were grown on medium containing sucrose, yeast, molasses, and agar under a 12 h light:12 h dark cycle at 25 °C. All studies were performed on randomly selected females aged 2–6 days post eclosion. Experimental flies were heterozygous for all transgenes and homozygous for either a wild-type or mutant (Hk) Hk allele, as indicated. Driver lines R23E10-GAL4, cry-GAL4, pdf-GAL4, OK167-GAL4, and GH146-GAL4 were used to target dFB neurons, cryptochrome- or PDF-expressing clock neurons, Kenyon cells, or olfactory projection neurons, respectively. Effector transgenes encoded a fluorescent marker for visually guided patch-clamp recordings (UAS-CD8:GFP); wild-type or mutant (Hk[R289M]) Hk rescue transgenes; an optical integrator of ROS exposure in the mitochondrial matrix (UAS-MitoTimer); the mitochondrial alternative oxidase AOX; wild-type or mutant (SOD1(AV4)) versions of human SOD1; catalase; or an N-myristoylated covalent hexamer (myr-MS6T2) of the singlet oxygen generator miniSOG; and RNAi constructs for interference with the expression of Hk, Sh, Shal, or cryptochrome (cry) (101402KK, 104474KK, 103363KK, and 7238GD or 105172KK, respectively; Vienna Drosophila Resource Center).

Sleep measurements. In standard sleep assays, females aged 3–5 days were individually inserted into 65-mm glass tubes, loaded into the Trinketics Drosophila Activity Monitor system, and housed under 12 h light:12 h dark conditions. Periods of inactivity lasting at least 5 min were classified as sleep. Immobile flies (<2 beam breaks per 24 h) were excluded from the analysis. In sleep deprivation experiments, a spring-loaded platform stacked with Trinketics monitors was slowly tilted by an electric motor, released, and allowed to snap back to its original position. The mechanical cycles lasted 12 s and were repeated continuously. Arousal thresholds in standard sleep assays were determined with the help of mechanical stimuli generated by vibration motors (Precision Microdrives, model 310-113). Stimuli were delivered once every hour, and the percentages of sleep-deprived flies awakened within 5 min of each 5-s stimulation episode were quantified.

Sleep after light-induced ROS generation was measured at zeitgeber time 9.5 h. Female flies aged 3–5 days and expressing miniSOG in dFB neurons were individually inserted into 35-mm glass tubes and loaded into a custom-built array of light-tight chambers. Each chamber was equipped with a high-power LED (Osmar Opto Semiconductor LB WSSM-FZHX, 35-0, 467 nm) running on an 80% duty cycle at 10 Hz and delivering 8 mW cm⁻² at the distal and 80 mW cm⁻² at the proximal end of the tube. In this intensity range, each miniSOG molecule in the central brain underwent an estimated 2–40 excitation cycles per second, based on the measured optical transmission of seven fly heads at 467 nm (4.8 ± 0.3% (mean ± s.e.m.), assumed to be isotropic) and a miniSOG absorption cross-section of 5.0 × 10⁻¹⁰ cm⁻².

The apparatus was operated in a temperature-controlled incubator (Sanyo MIR-154) at 25 °C. Excess heat from the high-power LEDs was removed by liquid heat exchangers (Thermo Electric Devices LI102), a centrifugal pump (RS 702-113), and a heat exchanger (Thermo Electric Devices LI102), Peltier module (Adaptive ETC-128-10-05-E), and CPU cooler (Corsair HEPS, 100 W). Signals from a high-speed amplifier (HCA-4M-101402KK, 104474KK, 103363KK, and 7238GD or 105172KK, respectively; Vienna Drosophila Resource Center). Statistical details, including test statistics, degrees of freedom, and exact P values (to four significant digits), are reported in Supplementary Tables 1, 2. All null hypothesis tests were two-sided. Group means were compared by two-sided t-test or one-way or two-way ANOVA, using repeated measures designs or the Welch correction where indicated, followed by planned pairwise post-hoc analyses using Holm–Sidak’s multiple comparisons test. Where the assumption of normality was not met, non-parametric equivalent tests were used. In multiple comparisons analyses, both Tukey’s and Sidak’s post-hoc tests were used. In cases where the means of slow inactivation time constants were poorly constrained, only the fast inactivation time constants were included in the analysis.
three times, on different days and with different batches of flies. The figures show representative examples.

**Reporting summary.** Further information on research design is available in the Nature Research Reporting Summary linked to this paper.

**Code availability**

Custom instrument control and analysis code used in this study is available from the corresponding author upon reasonable request.

**Data availability**

The datasets generated during this study are available from the corresponding author upon reasonable request.

Extended Data Fig. 1 | Chronic or acute dFB-restricted perturbations of redox chemistry have no effect on waking locomotor activity or arousability. a, Locomotor counts per waking minute of flies expressing R23E10-GAL4-driven SOD1 or pro-oxidant SOD1(A4V) do not differ from their respective parental controls (genotype effect: $P > 0.2612$, Kruskal–Wallis ANOVA with Dunn’s post-hoc test). b, The arousability of flies expressing R23E10-GAL4-driven SOD1 (left) or pro-oxidant SOD1(A4V) (right) does not differ from their respective parental controls (grey colours as in a) (genotype effects: $P > 0.2487$, vibrational force effects: $P < 0.0001$, vibrational force × genotype interactions: $P > 0.9857$, two-way ANOVA). Data are means ± s.e.m. of six trials per genotype ($n = 16–32$ flies each). c, Locomotor counts per waking minute of flies expressing R23E10-GAL4-driven miniSOG, with or without RNAi transgenes targeting Kv channel subunits, and parental controls, in a custom video-tracking system. Activity was monitored for 10 min before the photo-oxidation of miniSOG and then for a 30-min interval that included an initial 9-min exposure to blue light (genotype effect: $P = 0.0827$, illumination effect: $P = 0.8059$, illumination × genotype interaction: $P = 0.3086$, two-way repeated-measures ANOVA). Data are means ± s.e.m. $n$, number of flies (a, c) or trials (b). For statistical details see Supplementary Table 2.
Extended Data Fig. 2 | Chronic perturbations of redox chemistry in cryptochrome- or pigment dispersing factor (PDF)-expressing clock neurons, Kenyon cells, or olfactory projection neurons have no impact on sleep. a, Sleep in flies expressing cry-GAL4-driven SOD1 or SOD1(A4V) in clock neurons and parental controls. Kruskal–Wallis ANOVA with Dunn's post-hoc test failed to detect significant differences of experimental flies from both of their respective parental controls (P > 0.1426). b, Sleep in flies expressing pdf-GAL4-driven SOD1 or SOD1(A4V) in clock neurons and parental controls. Kruskal–Wallis ANOVA with Dunn's post-hoc test failed to detect significant differences of experimental flies from both of their respective parental controls (P > 0.1732). c, Sleep in flies expressing OK107-GAL4-driven SOD1 or SOD1(A4V) in KCs and parental controls. One-way ANOVA with Holm–Šidák's post-hoc test failed to detect significant differences of experimental flies from both of their respective parental controls (P > 0.0603). d, Sleep in flies expressing GH146-GAL4-driven SOD1 or SOD1(A4V) in olfactory projection neurons and parental controls. Kruskal–Wallis ANOVA with Dunn's post-hoc test failed to detect significant differences of experimental flies from both of their respective parental controls (P > 0.6901). Data are means ± s.e.m. n, number of flies. For statistical details see Supplementary Table 2.
Extended Data Fig. 3 | Chronic dFB-restricted manipulations of cryptochrome have no impact on sleep. Sleep in flies expressing two different R23E10-GAL4-driven cry-targeting RNAi transgenes and parental controls. One-way ANOVA with Holm-Šídák’s post-hoc test failed to detect significant differences of experimental flies from both of their respective parental controls ($P > 0.1718$). Data are means ± s.e.m. $n$, number of flies. For statistical details see Supplementary Table 2.
Extended Data Fig. 4 | Blue illumination of miniSOG-negative dFB neurons has no effect on their electrical activity. a–e, dFB neurons expressing R23E10-GAL4-driven CD8::GFP, before and after a 9-min exposure to blue light. Example voltage responses to current steps (a, sample sizes in b): illumination increases the input resistance (b, $R_m$; $P = 0.0098$, paired $t$-test) but not the membrane time constant (b, $\tau_m$; $P = 0.0723$, paired $t$-test) and leaves unchanged the current–spike frequency function (c, left; current × genotype interaction; $P = 0.9982$, two-way repeated-measures ANOVA) and interspike interval distribution (c, right; $P = 0.0947$, Kolmogorov–Smirnov test). Example $I_h$ (normalized to peak) evoked by voltage steps to $+40$ mV (d, sample sizes in e): illumination leaves unchanged the $I_h$ amplitude (e; $P = 0.8040$, Wilcoxon test) and both inactivation time constants (e, $\tau_{fast}$: $P = 0.6387$, $\tau_{slow}$: $P = 0.2958$, Wilcoxon tests). *$P < 0.05$. Data are means ± s.e.m. $n$, number of cells. For statistical details see Supplementary Table 2.
Extended Data Table 1 | Parameters of $I_h$ inactivation

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<tr>
<th>Condition</th>
<th>$\tau_{fast}$ (ms)</th>
<th>$\tau_{slow}$ (ms)</th>
<th>$A_{fast}/(A_{fast} + A_{slow})$</th>
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<tr>
<td>Before illumination</td>
<td>4.01 ± 0.72</td>
<td>27.18 ± 6.39</td>
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<td>After illumination</td>
<td>5.64 ± 0.67</td>
<td>38.91 ± 6.57</td>
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$R23E10 > \text{miniSOG}$

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<td>After illumination</td>
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$R23E10 > \text{CD8::GFP}$

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<td>$R23E10 &gt; \text{Hk(K289M)}$</td>
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<td>$R23E10 &gt; \text{SOD1(A4V)}$</td>
<td>6.30 ± 0.94</td>
<td>33.89 ± 9.32</td>
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Inactivation time constants of $I_h$ evoked by voltage steps to +40 mV were obtained by fitting double-exponential functions to the decaying phase of the current. $A_{fast}/(A_{fast} + A_{slow})$ represents the fraction of the fast component of the total $I_h$ current. Data are means ± s.e.m. n, number of cells. All dFB neurons expressed CD8::GFP in addition to the indicated transgenes.
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- A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
- The statistical test(s) used AND whether they are one- or two-sided
- A description of all covariates tested
- A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
- A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
- For null hypothesis testing, the test statistic (e.g. $F$, $t$, $r$) with confidence intervals, effect sizes, degrees of freedom and $P$ value noted. Give $P$ values as exact values whenever suitable.
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- For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
- Estimates of effect sizes (e.g. Cohen’s d, Pearson’s r), indicating how they were calculated

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- Video tracking system for sleep analysis: custom code in LabVIEW
- Electrophysiology: custom protocols in Nclamp/Neuromatic

Data analysis
- Two-photon imaging: custom script in MATLAB
- Video tracking system for sleep analysis: custom code in LabVIEW
- Electrophysiology: custom protocols in Neuromatic and Igor Pro
- Statistics: Prism

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Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

The datasets generated during this study are available from the corresponding author upon reasonable request.
Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

- Life sciences
- Behavioural & social sciences
- Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-flat.pdf

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

<table>
<thead>
<tr>
<th>Sample size</th>
<th>Sample sizes in behavioural experiments (typically n=32 flies per genotype) were chosen to detect 2-h differences in daily sleep with a power of 0.9.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Data exclusions</td>
<td>Immobile flies (&lt; 2 beam breaks per 24 h) in standard sleep assays in the Trikinetics Drosophila Activity Monitor system Flies sleeping before the onset of illumination in video-tracking analyses of sleep after light-induced ROS generation Flies with a confirmed waking time &lt;30 s in video-tracking analyses of waking movements after light-induced ROS generation Poorly constrained fits of slow inactivation time constants in voltage-clamp measurements of A-type currents</td>
</tr>
<tr>
<td>Replication</td>
<td>All behavioural experiments were run at least three times, on different days and with different batches of flies. The figures show representative examples.</td>
</tr>
<tr>
<td>Randomization</td>
<td>Female flies of a given genotype, as indicated in Methods and figure legends, were randomly selected for analysis.</td>
</tr>
<tr>
<td>Blinding</td>
<td>The investigators were blind to group allocation in MitoTimer imaging experiments but not otherwise.</td>
</tr>
</tbody>
</table>

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

<table>
<thead>
<tr>
<th>Materials &amp; experimental systems</th>
<th>Methods</th>
</tr>
</thead>
<tbody>
<tr>
<td>n/a</td>
<td>n/a</td>
</tr>
<tr>
<td>☒ Antibodies</td>
<td>☒ Involved in the study</td>
</tr>
<tr>
<td>☒ Eukaryotic cell lines</td>
<td>☒ ChIP-seq</td>
</tr>
<tr>
<td>☒ Palaeontology</td>
<td>☒ Flow cytometry</td>
</tr>
<tr>
<td>☒ Animals and other organisms</td>
<td>☒ MRI-based neuroimaging</td>
</tr>
<tr>
<td>☒ Human research participants</td>
<td></td>
</tr>
<tr>
<td>☒ Clinical data</td>
<td></td>
</tr>
</tbody>
</table>

Animals and other organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research

| Laboratory animals | Female Drosophila melanogaster strains, aged 2–6 days after eclosion, carrying transgenes, transgene combinations, or genomic mutations as indicated in Methods and figure legends. |
| Wild animals | n/a |
| Field-collected samples | n/a |
| Ethics oversight | No ethical approval was required for research on Drosophila melanogaster. |

Note that full information on the approval of the study protocol must also be provided in the manuscript.