Operation of a homeostatic sleep switch
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Sleep disconnects animals from the external world, at considerable risks and costs that must be offset by a vital benefit. Insight into this mysterious benefit will come from understanding sleep homeostasis: to monitor sleep need, an internal bookkeeper must track physiological changes that are linked to the core function of sleep.1 In *Drosophila*, a crucial component of the machinery for sleep homeostasis is a cluster of neurons innervating the dorsal fan-shaped body (dFB) of the central complex2,3. Artificial activation of these cells induces sleep, whereas reductions in excitability cause insomnia4,4. dFB neurons in sleep-deprived flies tend to be electrically active, with high input resistances and long membrane time constants, while neurons in rested flies tend to be electrically silent. Correlative evidence thus supports the simple view that homeostatic sleep control works by switching sleep-promoting neurons between active and quiescent states.5 Here we demonstrate state switching by dFB neurons, identify dopamine as a neuromodulator that operates the switch, and delineate the switching mechanism. Arousing dopamine6-10 caused transient hyperpolarization of dFB neurons within tens of milliseconds and lasting excitability suppression within minutes. Both effects were transduced by Dop1R2 receptors and mediated by potassium conductances. The switch to electrical silence involved the downregulation of voltage-gated A-type currents carried by Shaker and Shab, and the upregulation of voltage-independent leak currents through a two-pore-domain potassium channel that we term Sandman. Sandman is encoded by the CG8713 gene and translocates to the plasma membrane in response to dopamine. dFB-restricted interference with the expression of Shaker or Sandman decreased or increased sleep, respectively, by slowing the repetitive discharge of dFB neurons in the ON state or blocking their entry into the OFF state. Biophysical changes in a small population of neurons are thus linked to the control of sleep–wake state.

We recorded from dFB neurons (which were marked by *R23E10-GAL4 or R23E10-lexA*-driven green fluorescent protein (GFP) expression) while head-fixed flies walked or rested on a spherical treadmill. Because inactivity is a necessary correlate but insufficient proof of sleep, we restricted our analysis to awakening, which we defined as a locomotor bout after ≥5 min of rest10,11, during which the recorded dFB neuron had been persistently spiking. To deliver wake-promoting signals, we expressed the optogenetic actuator5,11 *CsChrimson* under *TH-GAL4* control in the majority of dopaminergic neurons, including the PPL1 and PPM3 clusters12, whose fan-shaped body (FB)-projecting members have been implicated in sleep control1,8. Illumination at 630 nm, sustained for 1.5 s to release a bolus of dopamine (Extended Data Fig. 1), effectively stimulated locomotion (32/38 trials; Fig. 1a, b). dFB neurons paused in successful (but not in unsuccessful) trials (Fig. 1a, b), and their membrane potentials dipped by 2–13 mV (7.50 ± 0.56 mV; mean ± standard error of the mean (s.e.m.) below the baseline during tonic activity (Fig. 1a, c). When flies bearing an undriven *CsChrimson* transgene were photostimulated, neither physiological nor behavioural changes were apparent (Fig. 1d–f). The tight correlation between the suppression of dFB neuron spiking and the initiation of movement (*P* < 0.0001, Fisher’s exact test) might, however, merely mirror a causal dopamine effect elsewhere, as *TH-GAL4* labels dopaminergic neurons throughout the brain12. Because localized dopamine applications to dFB neuron dendrites similarly caused awakening (see later), we consider this possibility remote.

Flies with enhanced dopaminergic transmission exhibit a short-sleeping phenotype that requires the presence of a D1-like receptor in dFB neurons6,11, suggesting that dopamine acts directly on these cells. dFB-restricted RNA interference (RNAi) confirmed this notion and pinpointed Dop1R2 as the responsible receptor (Fig. 2a), a conclusion reinforced by analysis of the mutant *Dop1R2Mo* allele.

Figure 1 | Optogenetic stimulation of dopaminergic neurons silences dFB neurons and promotes awakening. a, Membrane potential (black) of a dFB neuron and simultaneously recorded movement (blue) of a fly expressing *CsChrimson* in dopaminergic neurons. b, Spike rasters of dFB neurons in 59 trials. Photostimulation elicited a behavioural response in 32 trials (top) and no response in 6 trials (bottom). c, Individual (grey) and average (black) membrane potentials during the 32 trials with a behavioural response. Spikes are blanked for clarity. d, Membrane potential (black) of a dFB neuron and simultaneously recorded movement (blue) of a fly lacking *CsChrimson* expression in dopaminergic neurons. e, Spike rasters of dFB neurons in 59 trials. Photostimulation elicited a behavioural response in 2 trials (top) and no response in 57 trials; of these, 56 were randomly selected for display (bottom). f, Individual (grey) and average (black) membrane potentials during the 57 trials without a behavioural response. Spikes are blanked for clarity.

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Figure 2 | Dopamine inhibits dFB neurons via Dop1R2 and the transient opening of a potassium conductance. a, Sleep in flies expressing R23E10-GAL4-driven RNAi targeting dopamine receptor transcripts and parental controls (circles, individual flies; horizontal lines, group means). One-way analysis of variance (ANOVA) detected a significant genotype effect ($P < 0.0001$); red indicates a significant difference from both parental controls in pairwise post-hoc comparisons. b, R23E10-GAL4-driven CD8::GFP expression in dFB neurons (top). Placement of pipettes for whole-cell recording and pharmacological stimulation (bottom). c, Membrane potentials of dFB neurons after a 250 ms pulse of dopamine, in control conditions of low intracellular chloride (1 mM, black, top and bottom); in cells expressing R23E10-GAL4-driven RNAi targeting Dop1R2 (red, top); in the presence of 2 μg ml$^{-1}$ intracellular PTX (blue, top); in elevated intracellular chloride (141 mM, light grey, bottom); and in intracellular caesium (140 mM, dark grey, bottom). Traces are averages of five dopamine applications.

(Extended Data Fig. 2a–c). Previous evidence that Dop1R1, a receptor not involved in regulating baseline sleep$^8$, confers responsiveness to dopamine when expressed in the dFB$^{14}$ indicates that either D1-like receptor can fulfil the role normally played by Dop1R2. Loss of Dop1R2 increased sleep during the day and the late hours of the night, by prolonging sleep bouts without affecting their frequency (Extended Data Fig. 2a, d, e). This sleep pattern is consistent with reduced sensitivity to a dopaminergic arousal signal.

To confirm the identity of the effective transmitter, avoid dopamine release outside the dFB, and reduce the transgene load for subsequent experiments, we replaced optogenetic manipulations of the dopaminergic system with pressure ejections of dopamine onto dFB neuron dendrites (Fig. 2b). Like optogenetically stimulated secretion, focal application of dopamine hyperpolarized the cells and suppressed their spiking (Fig. 2c and Extended Data Fig. 3a, b). The inhibitory responses could be blocked at several nodes of an intracellular signalling pathway that connects the activation of dopamine receptors to the opening of potassium conductances (Fig. 2c and Extended Data Fig. 3b); by RNAi-mediated knockdown of Dop1R2; by the inclusion in the patch pipette of pertussis toxin (PTX), which inactivates heterotrimeric G proteins of the $G_{i/o}$ family$^{15}$; and by replacing intracellular potassium with caesium, which obstructs the pores of G-protein–coupled inward-rectifier channels$^{14}$. Elevating the chloride reversal potential above resting potential left the polarity of the responses unchanged (Fig. 2c and Extended Data Fig. 3b), corroborating that potassium conductances mediate the bulk of dopaminergic inhibition.

Coupling of Dop1R2 to $G_{i/o}$, although documented in a heterologous system$^{15}$, represents a sufficiently unusual transduction mechanism for a predicted D1-like receptor to prompt us to verify its behavioural relevance. Like the loss of Dop1R2, temperature-inducible expression of PTX in dFB neurons increased overall sleep time by extending sleep bout length (Extended Data Fig. 2f, g).

While a single pulse of dopamine transiently hyperpolarized dFB neurons and inhibited their spiking, prolonged dopamine applications (50 ms pulses at 10 Hz, or 20 Hz optogenetic stimulation, both sustained for 2–10 min) switched the cells from electrical excitability (ON) to quiescence (OFF) (Fig. 3a–c and Extended Data Fig. 4a–c). The switching process required dopamine as well as Dop1R2 (Fig. 3b, c), but once the switch had been actuated the cells remained in the OFF state—and flies, awake (Fig. 3d)—without a steady supply of transmitter. Input resistances and membrane time constants dropped to 53 ± 1.8 and 24.0 ± 1.3% of their initial values (means ± s.e.m., $n = 15$ cells; Fig. 3b, c), and depolarizing currents no longer elicited action potentials (15 out of 15 cells) (Fig. 3a and Extended Data Fig. 4a). The biophysical properties of single dFB neurons, recorded in the same individual before and after operating the dopamine switch, varied as widely as those in sleep-deprived and rested flies$^4$.

Dopamine-induced changes in input resistance and membrane time constant occurred from similar baselines in all genotypes (Extended Data Fig. 5a, b) and followed single-exponential kinetics with time constants of 1.07–1.10 min (Fig. 3b, c). The speed of conversion points to post-translational modification and/or translocation of ion channels between intracellular pools and the plasma membrane as the underlying mechanism(s). In 7 out of 15 cases, we held recordings long enough to observe the spontaneous recommencement of spiking (Fig. 3a, d), which was accompanied by a rise to baseline of input resistance and membrane time constant, after 7–60 min of quiescence (mean ± s.e.m. = 25.86 ± 7.61 min). The temporary suspension of electrical output is thus part of the normal activity cycle of dFB neurons and not a dead end brought on by our experimental conditions.

dFB neurons in the ON state expressed two types of potassium current: voltage-dependent A-type$^{16}$ and voltage-independent non-A-type currents (Fig. 3e–g and Extended Data Fig. 6a–c). The current–voltage (I–V) relation of $I_A$, resembled that of Shaker, the prototypical A-type channel$^{17,18}$; no current flowed below ~50 mV, the approximate voltage threshold of Shaker$^{17,18}$, above ~40 mV, peak currents increased steeply with voltage (Fig. 3e, f) and inactivated with a time constant$^{18}$ of 7.5 ± 2.1 ms (mean ± s.e.m., $n = 7$ cells; Extended Data Fig. 6c, d). Non-A-type currents showed weak outward rectification with a reversal potential of ~80 mV (Fig. 3e, g), consistent with potassium as the permeant ion, and no inactivation (Extended Data Fig. 6b).

Switching the neurons OFF changed both types of potassium current. $I_A$ diminished by one-third (Fig. 3e, f), whereas $I_{non-A}$ A nearly quadrupled when quantified between resting potential and spike threshold (Fig. 3g). The weak rectification of $I_{non-A}$ in the ON state vanished in the OFF state, giving way to the linear I–V relationship of an ideal leak conductance (Fig. 3e, g). dFB neurons thus upregulated $I_L$ in the sleep-promoting ON state (Fig. 3e, f). When dopamine switches the cells OFF, voltage-dependent currents are attenuated and leak currents augmented (Fig. 3e–g). This seesaw form of regulation should be sensitive to perturbations of the neurons’ ion channel inventory: depletion of voltage-gated A-type (Kv) channels (which predominate in the ON state) should tip the cells towards the OFF state; conversely, loss of leak channels (which predominate in the OFF state) should favour the ON state. To test these predictions, we examined sleep in flies carrying R23E10-GAL4-driven RNAi transgenes for dFB-restricted interference with individual potassium channel transcripts.

RNAi-mediated knockdown of two of the five Kv channel types of Drosophila$^{19}$ (Shaker and Shab) reduced sleep relative to parental controls, while knockdown of the remaining three types had no effect (Fig. 4a). Biasing the potassium channel repertoire of dFB neurons against A-type conductances thus tilts the neurons’ excitable state towards quiescence (Fig. 4b–f), causing insomnia (Fig. 4a), but leaves transient and sustained dopamine responses unaffected (Fig. 4e–g and Extended Data Fig. 3b). The seemingly counterintuitive conclusion that reducing a potassium current would decrease, not increase, action potential discharge is explained by a requirement for A-type channels in generating repetitive activity$^{16,20}$ of the kind displayed by dFB neurons during sleep (Fig. 1). Depleting Shaker from dFB neurons...
shifted the interspike interval distribution towards longer values (Fig. 4d), as would be expected if Kv channels with slow inactivation kinetics replaced rapidly inactivating Shaker as the principal force opposing the generation of the next spike. These findings identify a potential mechanism for the short-sleeping phenotypes caused by mutations in Shaker21, its β subunit Hyperkinetic22, or its regulator sleepless23 (Extended Data Fig. 7).

Leak conductances are typically formed by two-pore-domain potassium (K2P) channels24, dFB-restricted RNAi of one member of the 11-strong family of Drosophila K2P channels25, encoded by the CG8713 gene, increased sleep relative to parental controls; interference with the remaining 10 K2P channels had no effect (Fig. 4a). Recordings from dFB neurons after knockdown of the CG8713 gene product, which we term Sandman, revealed undiminished non-A-type currents in the ON state (Fig. 4c) and intact responses to a single pulse of dopamine (Fig. 4g and Extended Data Fig. 4a) but a defective OFF switch: during prolonged dopamine exposure (Fig. 4b), voltage-gated sodium channels remain functional in the OFF state. The difficulty of driving control cells to action potential threshold in this state (Fig. 3a and Extended Data Fig. 4a) must therefore be due to a lengthening of electrotonic distance between sites of current injection and spike generation. This lengthening is an expected consequence of a current leak, which may uncouple the axonal spike generator from somatodendritic synaptic inputs or pacemaker currents when sleep need is low.

The two kinetically and mechanistically distinct actions of dopamine on dFB neurons—instant, but transient, hyperpolarization and a similarly disabled OFF switch (Fig. 4c, e, f). This, combined with the absence of detectable Sandman currents in the ON state (Fig. 4c), suggests that Sandman is internalized in electrically active cells and recycled to the plasma membrane when dopamine switches the neurons OFF.

Because dFB neurons lacking Sandman spike persistently even after prolonged dopamine exposure (Fig. 4b), voltage-gated sodium channels remain functional in the OFF state. The difficulty of driving control cells to action potential threshold in this state (Fig. 3a and Extended Data Fig. 4a) must therefore be due to a lengthening of electrotonic distance between sites of current injection and spike generation. This lengthening is an expected consequence of a current leak, which may uncouple the axonal spike generator from somatodendritic synaptic inputs or pacemaker currents when sleep need is low.

The two kinetically and mechanistically distinct actions of dopamine on dFB neurons—instant, but transient, hyperpolarization and a delayed, but lasting, switch in excitable state—ensure that transitions to vigilance can be both immediate and sustained, providing speedy alarm responses and stable homeostatic control. The key to stability lies in the switching behaviour of dFB neurons, which is
Figure 4 | The targets of antagonistic modulation by dopamine—
Shaker and Sandman—have opposing effects on sleep. a, Sleep in flies expressing R23E10-GAL4-driven RNAi targeting K_V or K_O channels and parental controls (circles, individual flies; horizontal lines, group means). One-way ANOVA detected significant genotype effects (P < 0.0001 for K_V channels; P < 0.0001 for K_O channels); green and blue colours indicate significant differences from both parental controls in pairwise post-hoc comparisons. b, Voltage responses of two dFB neurons to current steps, before and after the application of dopamine. The neurons expressed R23E10-GAL4-driven RNAi targeting Shaker (green, top) or Sandman (blue, bottom). c, Amplitudes of I_sh from 40 mV (left) and I_Soden at −40 mV (right) in controls (black, n = 7 cells), neurons expressing R23E10-GAL4-driven RNAi targeting Shaker (green, n = 7 cells) or Sandman (blue, n = 8 cells), and in the presence of 1.5 μg mL⁻¹ intracellular BoNT/C (orange, n = 8 cells), in the ON state (open symbols) and after dopamine-induced switching to the OFF state (red filled symbols). Data are means ± s.e.m. Two-way repeated-measures ANOVA detected significant interaction between experimental condition and neuronal state for I_Soden (P = 0.0018). I_sh was reduced in cells expressing ShakerRNAi relative to all other groups (P = 0.0409). I_Soden differed between ON and OFF states in controls (P = 0.0005) and cells expressing ShakerRNAi (P = 0.0003), but not in cells expressing SandmanRNAi (P = 0.9119) or containing BoNT/C (P = 0.9119); I_Soden in the ON state did not differ among groups (P = 0.0782). d, Frequency and cumulative frequency distributions (inset of interspike intervals (ISIs) in controls (black) and neurons expressing R23E10-GAL4-driven RNAi targeting Shaker (green) or Sandman (blue)). The interspike interval distribution of neurons expressing ShakerRNAi differed from that of the other groups (P < 0.0001 for both comparisons; Kolmogorov–Smirnov test). e, f, Time courses of changes in input resistance (R_in) and membrane time constant (τ_m) during the application of dopamine, in controls (black, n = 15 cells), neurons expressing R23E10-GAL4-driven RNAi targeting Shaker (green, n = 6 cells) or Sandman (blue, n = 7 cells), and in the presence of 1.5 μg mL⁻¹ intracellular BoNT/C (orange, n = 8 cells). Data are means ± s.e.m. Two-way repeated-measures ANOVA detected a significant interaction between time and experimental condition (P < 0.0001 for R_in; P < 0.0001 for τ_m). dFB neurons expressing SandmanRNAi or containing BoNT/C differed from controls (P < 0.0001 for all pairwise comparisons), but flies expressing ShakerRNAi did not (P = 0.9993 for R_in; P = 0.8743 for τ_m). g, Membrane potentials of dFB neurons after a 250 ms pulse of dopamine, in control flies (black), flies expressing R23E10-GAL4-driven RNAi targeting Shaker (green) or Sandman (blue), and in the presence of 1.5 μg mL⁻¹ intracellular BoNT/C (orange). Traces are averages of five dopamine applications.

Online Content Methods, along with any additional Extended Data display items and Source Data, are available in the online version of the paper; references unique to these sections appear only in the online paper.

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Author Contributions D.P., J.M.D. and G.M. designed the study and analysed the results. All electrophysiological recordings were done by D.P.; J.M.D. performed molecular manipulations and behavioural analyses with the help of S.M.S. and A.J.F.T. C.B.T. developed instrumentation. G.M. wrote the paper.

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**METHODS**

**Drosophila strains and culture.** Driver lines R23E10-GAL4 or R23E10-lexa and TH-GAL4 (ref. 32) were used to target dFB neurons and dopaminergic neurons, respectively. Effector transgenes encoded fluorescent markers for visually guided patch-clamp recordings (UAS-Cd8::GFP33 and lexAop-CD2::GFP34); a temperature-inducible system34 for the expression of pertussis toxin38 (UAS-PTX; tubP-GAL80); the optogenetic activator CsChrimson35; and RNAi constructs33, along with UAS-Dex2, to interfere with the expression of the dopamine receptors DopLP1 (105324K); DopLP2 (147172D) and DopECR (103494K); the Kc channels Shaker (104474K), Shal (201228K), Shal (103363K), Kp (100589KD) and the K_5p channels Task7 (8565GD), Task6 (9073GD), Ork1 (104883KD), CG11688 (8302GD), CG34396 (100436K), CG43155 (101483K), CG42594 (46154G), CG8713 (47977GD), CG9194 (110628K) and CG24340 (104521K). Codes in parentheses identify transformations in the GD and KK libraries of the Vienna Drosophila Resource Center. The genotype of control flies in electrophysiological experiments was w^{1118}; UAS-Cd8::GFP, R23E10-GAL4.

Fly stocks were grown on media of sucrose, yeast, molasses and agar under a 12 h light:12 h dark cycle at 25°C unless they expressed GAL80; in this case the experimental animals and all relevant controls were grown at 18°C. Flies expressing CsChrimson were transferred to food supplemented with 2 mM all-trans retinal upon eclosion. All studies were performed on animals aged 3–10 days. Flies were routinely sleep-deprived39 for >12 h before electrophysiological recordings to increase the likelihood of finding dFB neurons in the electrically active ON state after break-in.

**Movement tracking, electrophysiology and optogenetics.** Male and female flies with a dorsal cranial window were head-fixed to a custom mount, using thermoplastic wax with a melting point of 32°C (Agar Scientific), and placed on a spherical treadmill40,41. The treadmill consisted of an air-supported trackball made of extruded styrofoam (13 mm diameter; 50 mg) in a 14 mm tube. An image of a small region of the ball's surface under 640 nm LED illumination was relayed onto the sensor of an optical mouse (Logitec M-U0017). The sensor was interfaced with a microcontroller board (Arduino Due) based on the Atmel SAM3X CPU and read out in real time using the onboard D/A converter. The resolution of the readout corresponds to 4 m s^{-1} increments in the tangential speed of the trackball.

The brain was continuously superfused with extracellular solution equilibrated with 95% O_2, 5% CO_2 and containing 103 mM NaCl, 1 mM KCl, 5 mM TRIS, 8 mM trehalose, 10 mM glucose, 7 mM sucrose, 2.6 mM NaHCO_3, 1 mM Na_2HPO_4, 1.5 mM CaCl_2, 4 mM MgCl_2, pH 7.3. Somata of GFP-labelled dFB neurons were visually targeted with borosilicate glass electrodes (7–13 MΩ) and custom procedures in Igor Pro (WaveMetrics) and MATLAB (The MathWorks). Movement tracking, electrophysiology and optogenetics.

**Sleep measurements.** Female flies were individually inserted into 65 mm glass tubes, loaded into the Trikinetics Drosophila Activity Monitor system, and housed under 12 h light:12 h dark conditions. Periods of inactivity (no beam breaks) lasting at least 5 min were classified as sleep38,10. Immobile flies (<2 beam breaks per 24 h) were excluded from analysis. Group sizes for sleep measurements (typically n = 16 flies; in some cases multiples of 16) reflect the capacity of the Trikinetics Drosophila Activity Monitors, which were designed to accommodate 16 experimental flies along with 16 controls.

**Statistics.** Data were analysed in Prism 6 (GraphPad). Group means were compared by one-way or two-way ANOVA, using repeated measures designs where appropriate, followed by planned pairwise post-hoc analyses using Holm–Šidak's multiple comparisons test. Where the assumptions of normality or sphericity were violated (as indicated by Shapiro–Wilk and Brown–Forsythe tests, respectively), group means were compared by two-sided Mann–Whitney or Kruskal–Wallis tests, the latter followed by Dunn's multiple comparisons test. Contingencies between the suppression of dFB neuron activity and awakening were analysed by Fisher's exact test. Interspike interval distributions were evaluated by Kolmogorov–Smirnov test, using the Bonferroni correction to adjust the level of statistical significance. No statistical methods were used to predetermine sample sizes. The experiments were not randomized, and the investigators were not blinded to allocation during experiments and outcome assessment.

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Extended Data Figure 1 | Optogenetic stimulation of dopaminergic neurons. Dopaminergic neurons expressing CsChrimson under TH-GAL4 control were driven with 3 ms pulses of 630 nm light at the indicated frequencies. Optical power at the sample was ~28 mW cm$^{-2}$.

a, Examples of voltage responses to optical pulse trains. b, The ratio of light-evoked action potentials to optical pulses was close to 1 at driving frequencies between 5 and 20 Hz ($n=36$ trials on 6 cells). Data are means ± s.e.m.
Extended Data Figure 2 | Changes in sleep after interference with Dop1R2 signalling are consistent with diminished sensitivity to arousing dopamine. a, Sleep during a 24 h day in homozygous carriers of the Dop1R2<sup>MB05108</sup> allele (red, n = 32 flies) and heterozygous controls (black, n = 31 flies). Data are means ± s.e.m. Two-way repeated-measures ANOVA detected a significant interaction between time of day and genotype (P < 0.0001). b, Sleep during a 24 h day in homozygous carriers of the Dop1R2<sup>MB05108</sup> allele (red, n = 28 flies) and heterozygous controls (black, n = 32 flies). Data are means ± s.e.m. Two-way repeated-measures ANOVA failed to detect a significant interaction between time of day and genotype (P = 0.4736). c, Sleep in homozygous and heterozygous carriers of the Dop1R2<sup>MB05108</sup> or Dop1R2<sup>MB05108</sup> alleles (circles, individual flies; horizontal lines, group means). Mann–Whitney tests detected a significant effect of the Dop1R2<sup>MB05108</sup> allele (P = 0.0219, red), but not of the Dop1R2<sup>MB05108</sup> allele (P = 0.6750). The Dop1R2<sup>MB05108</sup> allele contains a transposon insertion in a non-coding region of the Dop1R2 gene, which reduces mRNA levels in homozygous carriers by only 14% (ref. 4), thus explaining the lack of a phenotype. The inability of Dop1R2<sup>MB05108</sup> to suppress the short-sleeping phenotype of flies with enhanced dopaminergic transmission<sup>4</sup> therefore does not argue against a role of Dop1R2 in the dFB. d, Sleep during a 24 h day in flies expressing R23E10-GAL4-driven RNAi targeting Dop1R2 (red, n = 48 flies) and parental controls (open symbols: R23E10-GAL4, n = 48 flies; filled symbols: undriven UAS-Dop1R2<sup>RNAi</sup>, n = 32 flies). Data are means ± s.e.m. Two-way repeated-measures ANOVA detected a significant interaction between time of day and genotype (P < 0.0001). e, Average length of daytime sleep bouts in flies expressing R23E10-GAL4-driven RNAi targeting Dop1R2 and parental controls. Data are means ± s.e.m. One-way ANOVA detected a significant genotype effect (P = 0.0015); red indicates a significant difference from both parental controls in pairwise post-hoc comparisons. f, Sleep in flies with temperature-inducible R23E10-GAL4-driven expression of PTX and parental controls (circles, individual flies; horizontal lines, group means). Two-way ANOVA detected a significant interaction between genotype and temperature (P = 0.0002); blue indicates a significant increase upon switching from non-inducing to inducing temperatures in pairwise post-hoc comparisons. g, Average length of daytime sleep bouts in flies with temperature-inducible R23E10-GAL4-driven expression of PTX and parental controls. Data are means ± s.e.m. Two-way ANOVA detected a significant interaction between genotype and temperature (P = 0.0002); blue indicates a significant increase upon switching from non-inducing to inducing temperatures in pairwise post-hoc comparisons.
Extended Data Figure 3 | Dopamine hyperpolarizes dFB neurons and inhibits their spiking. a, Membrane potential of a dFB neuron during a 250 ms pulse of dopamine. b, Average amplitude of hyperpolarization evoked by dopamine in the indicated numbers of cells. Data are means ± s.e.m. Kruskal–Wallis test detected a significant difference between groups (P < 0.0001); asterisks indicate significant differences from controls in pairwise post-hoc comparisons.
Extended Data Figure 4 | Optogenetic stimulation of dopaminergic neurons switches dFB neurons to quiescence. Flies expressing CsChrimson under TH-GAL4 control in dopaminergic neurons were photostimulated with 3 ms pulses of 630 nm light at 20 Hz. a, Voltage responses to current steps were recorded in the same cell, before and after optogenetic stimulation of dopaminergic neurons (black and red traces). Red and grey traces in the OFF state (right) indicate current injections matching or exceeding those in the ON state, respectively (left). b, c, Time courses of changes in input resistance ($R_m$) and membrane time constant ($\tau_m$) of dFB neurons during optogenetic stimulation of dopaminergic neurons ($n = 7$ cells). Data are means ± s.e.m. One-way repeated-measures ANOVA detected significant effects of time ($P = 0.0135$ for $R_m$; $P = 0.0222$ for $\tau_m$).
Extended Data Figure 5 | Membrane properties of dFB neurons in the ON state. a, Input resistances ($R_m$) of the indicated numbers of cells. Data are means ± s.e.m. Kruskal–Wallis test failed to detect a significant difference between groups ($P = 0.8997$). b, Membrane time constants ($\tau_m$) of the indicated numbers of cells. Data are means ± s.e.m. Kruskal–Wallis test failed to detect a significant difference between groups ($P = 0.1682$).
Extended Data Figure 6 | Measurements of potassium currents in voltage clamp. a, Voltage steps from a holding potential of −110 mV (top) elicited the full complement of potassium currents expressed by a dFB neuron (I_{total}, bottom). b, Stepping the same neuron from a holding potential of −30 mV (top) elicited potassium currents lacking the A-type component (I_{non-A}, bottom). c, Digital subtraction of I_{non-A} (b, bottom) from I_{total} (a, bottom) yielded an estimate of I_A. Note the expanded timescale. d, Individual (grey) and average (black) A-type currents of seven dFB neurons, evoked by step depolarization to 40 mV. The magenta line represents a single-exponential fit to the average.
Extended Data Figure 7 | Loss of Shaker and its interacting partners, Hyperkinetic and Sleepless, from dFB neurons has similar effects on sleep. Sleep in flies expressing R23E10-GAL4-driven RNAi targeting Shaker, Hyperkinetic or sleepless and parental controls (circles, individual flies; horizontal lines, group means). One-way ANOVA detected a significant genotype effect ($P < 0.0001$); green indicates significant differences from both parental controls in pairwise post-hoc comparisons.