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Chronic social isolation signals starvation and reduces sleep in *Drosophila*

https://doi.org/10.1038/s41586-021-03837-0

Received: 1 May 2020

Accepted: 20 July 2021

Published online: 18 August 2021

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Social isolation and loneliness have potent effects on public health[1–4]. Research in social psychology suggests that compromised sleep quality is a key factor that links persistent loneliness to adverse health conditions[5,6]. Although experimental manipulations have been widely applied to studying the control of sleep and wakefulness in animal models, how normal sleep is perturbed by social isolation is unknown. Here we report that chronic, but not acute, social isolation reduces sleep in *Drosophila*. We use quantitative behavioural analysis and transcriptome profiling to differentiate between brain states associated with acute and chronic social isolation. Although the flies had uninterrupted access to food, chronic social isolation altered the expression of metabolic genes and induced a brain state that signals starvation. Chronically isolated animals exhibit sleep loss accompanied by overconsumption of food, which resonates with anecdotal findings of loneliness-associated hyperphagia in humans. Chronic social isolation reduces sleep and promotes feeding through neural activities in the peptidergic fan-shaped body columnar neurons of the fly. Artificial activation of these neurons causes misperception of acute social isolation as chronic social isolation and thereby results in sleep loss and increased feeding. These results present a mechanistic link between chronic social isolation, metabolism, and sleep, addressing a long-standing call for animal models focused on loneliness[7].

Fruit flies are social animals[8,9], and exhibit dynamic social network structures and collective behaviours, which contribute to environmental sensing, foraging, feeding, fighting, mating, oviposition, circadian time setting and even the existence of 'culture'[10,11]. These important aspects of social interactions imply that insects can provide suitable models for studying how the objective absence of social relationships is perceived and represented in the brain.

Social experience affects sleep need in *Drosophila*[12]. Here we revisited this finding by exploring how social isolation affects sleep in flies that have prior social experience. We tested sleep behaviour after maintaining 1, 2, 5, 25 or 100 male flies in a food vial for 7 days. Group-housed flies, regardless of group size (2, 5, 25 or 100), exhibited similar sleep profiles. By contrast, flies housed in isolation displayed a significant loss of sleep, mainly distributed during the daytime (Extended Data Fig. 1, Supplementary Information).

Acute versus chronic social isolation

We next manipulated the duration of social isolation: flies were either isolated or housed in a group of 25 flies for 1, 3, 5, or 7 days, before sleep was measured in a *Drosophila* activity monitor (DAM) (Fig. 1a). Sleep profiles, which display the proportion of time spent sleeping in consecutive 30-min segments over 24 h, showed that chronic social isolation (5 or 7 days; Fig. 1d, e) changed sleep architecture primarily during the daytime and especially during an interval of several hours following dawn (lights on). Although short durations of social isolation (1 or 3 days; Fig. 1b, c) did not induce sleep loss, chronic social isolation (5 or 7 days) significantly reduced daily total sleep, daytime sleep and sleep between Zeitgeber time (ZT) 0 and ZT4 (corresponding to the first 4 h after lights-on in a light–dark (LD) cycle) (Fig. 1f–i).

To assess how social isolation alters daytime sleep, we pooled all daytime sleep bouts from all animals tested for a given condition and plotted their distributions as cumulative relative fractions for bout lengths. Acute social isolation (1 day) produced sleep bout distributions that were indistinguishable from those of 1-day group-housed flies (Fig. 1j, Extended Data Fig. 2a). Flies that were socially isolated for 3 days showed slightly different sleep bout distributions from their group-housed counterparts (Fig. 1k, Extended Data Fig. 2b). However, there was no deficit in total daily sleep, daytime sleep or ZT0–4 sleep in these flies (Fig. 1g). Flies that were isolated for longer periods (5 or 7 days) had sleep distributions that were significantly different from those of their group-housed counterparts (Extended Data Fig. 2c, d). Cumulative relative frequency curves of daytime sleep bouts from chronically isolated flies climbed faster than those of their group-housed counterparts as shorter sleep bouts accumulated (5 or 7 days Fig. 1l, m). Over seven days of isolation, daily total sleep, daytime sleep and ZT0–4 sleep all decreased progressively (Fig. 1n–p).

We also used age-matched flies to rule out the possibility that chronic social isolation induced sleep loss because the flies were older (Extended Data Fig. 3a–e). Chronic social isolation (7 days) induced

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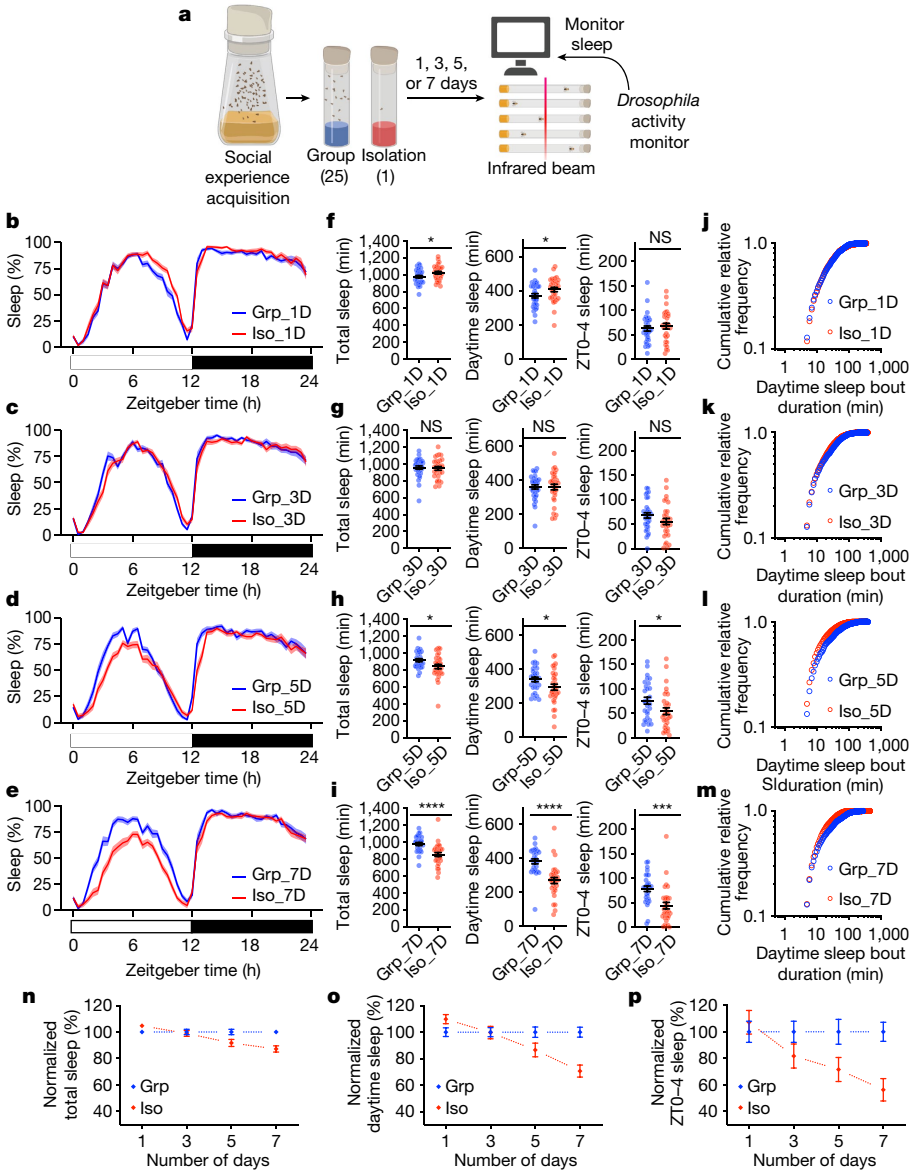

Fig. 1 | Sleep is reduced by chronic but not acute social isolation in *Drosophila*. **a**, Measuring sleep using *Drosophila* activity monitors after 1, 3, 5 or 7 days of group enrichment or social isolation. **b**–**e**, Sleep profiles (average proportion of time spent sleeping in consecutive 30-min segments during a 24-h LD cycle; mean ± s.e.m.) of flies after 1 (**b**), 3 (**c**), 5 (**d**) and 7 (**e**) days of group enrichment or social isolation. **f**–**i**, Quantification (mean ± s.e.m. with individual data points) of daily total sleep, daytime sleep and ZT0–4 sleep for flies after 1 (**f**), 3 (**g**), 5 (**h**) and 7 (**i**) days of group enrichment or social isolation. **j**–**m**, Plots of cumulative relative frequency (CRF) for distributions of daytime sleep bout durations for flies after 1(**j**), 3 (**k**), 5 (**l**) and 7 (**m**) days of group enrichment or social isolation (see Extended Data Fig. 2a–d for density plots of the same data). **n**–**p**, Normalized (mean ± s.e.m.) daily total sleep (**n**), daytime sleep (**o**), and ZT0–4 sleep (**p**) in isolated or group-housed flies over days 1–7. For each duration (1, 3, 5 or 7 days) of group enrichment or social isolation, sleep parameters for socially isolated animals were normalized to their group-treated counterparts. **b**–**i**, **n**–**p**, $n = 29$–32 flies; two-sided unpaired *t*-tests with Welch's correction; $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$; NS, not significant. For *n* and *P* values, see Source Data.

sleep loss consistently in various isogenic strains, in aged (4-week-old) wild-type flies, and in sleep inbred panel (SIP) strains with different baseline levels of sleep (Extended Data Figs. 3, 4, Supplementary Information).

Chronic social isolation induces hunger

We prepared RNA sequencing (RNA-seq) libraries for three conditions: socially enriched flies (group treated, Grp), chronically isolated flies (isolated for 7 days, Iso_7D) and acutely isolated flies (isolated for 1 day, Iso_1D). Raster plots demonstrate sleep bouts of individual animals during a 24-h period measured immediately after group enrichment or social isolation. Daytime sleep was reduced and much more fragmented in chronically isolated flies than in group-housed or acutely isolated flies (Fig. 2a–c). We collected fly heads between ZT0.5 and ZT2 (grey bars in Fig. 2a–c), a window of time within ZT0–4 that immediately preceded significant loss of daytime sleep in chronically isolated flies. Using differential gene expression analyses, intersectional and clustering strategies, we identified candidate genes for the sleep loss induced by chronic social isolation. These 214 candidate genes showed differences in expression in chronically isolated flies compared with both acutely isolated and group-housed flies and underwent unidirectional changes during the process of chronic social isolation (Extended Data Fig. 5a–d, Supplementary Information). Gene ontology enrichment

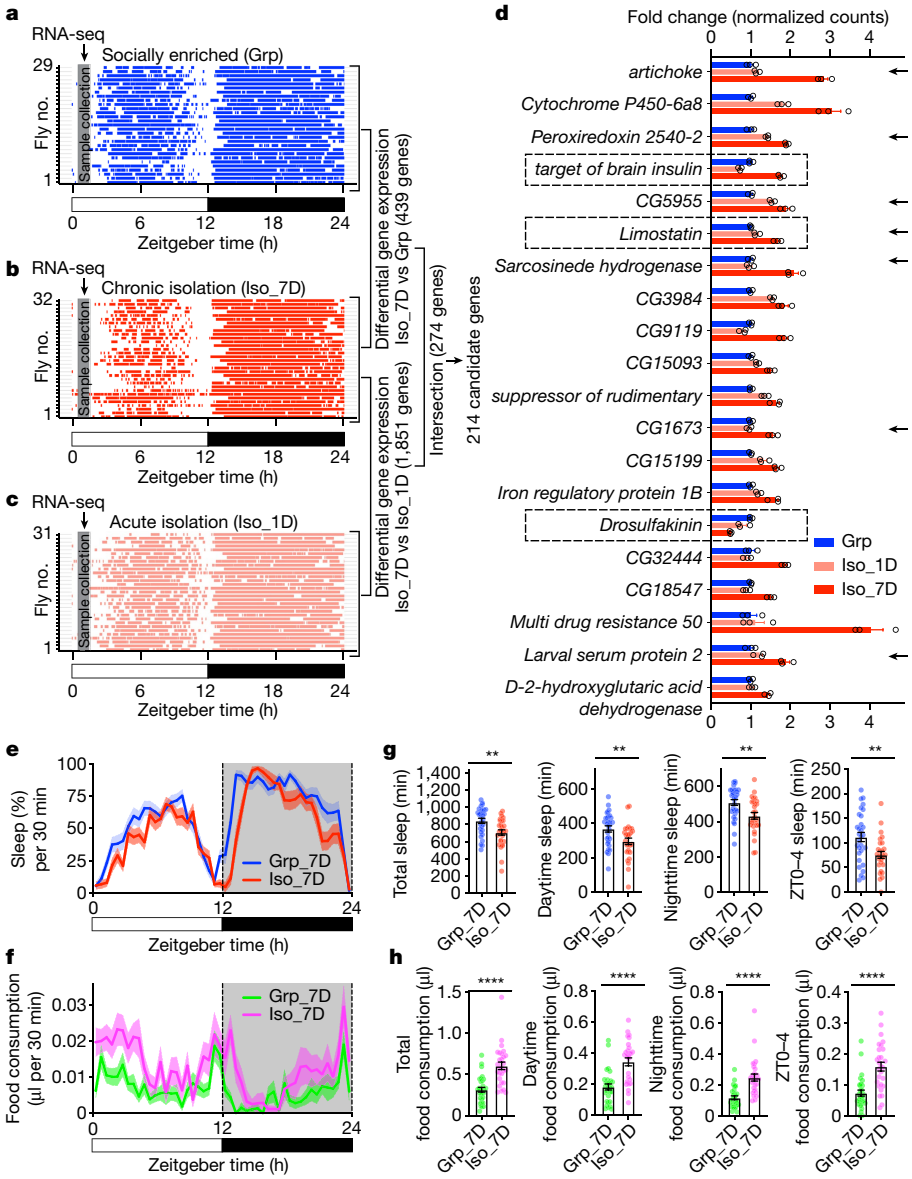

Fig. 2 | Chronic social isolation induces a starvation gene expression program and results in excessive feeding. a–**c**, Raster plot of sleep bouts of individual flies after group enrichment (Grp) (**a**), acute social isolation (Iso_1D) (**c**) and chronic social isolation (Iso_7D) (**b**). Each row is an individual fly; each coloured bar represents a sleep bout within a 24-h LD cycle. Grey vertical bars indicate the time window (ZT0.5–2) during which fly heads were collected for RNA-seq. For each condition, 29–32 representative flies are shown. Differential gene expression analyses were conducted between Iso_7D and Grp and between Iso_7D and Iso_1D. We identified 274 genes within the intersection of these two comparisons and 214 candidate genes using a clustering approach (Extended Data Fig. 5, Supplementary Information). **d**, Fold changes (mean ± s.e.m.) of normalized counts of the top 20 candidate genes (ranked by adjusted *P* value in the comparison of Iso7 versus Grp; $n = 3$ samples). Arrows, genes that are regulated after 24 h starvation[16]. **e**, **f**, Sleep (**e**) and feeding (**f**) measured by ARC assay in flies after 7 days of group enrichment or social isolation. Sleep profile, mean ± s.e.m. average proportion of time spent sleeping in consecutive 30-min segments during 24-h LD cycle. Feeding profile, mean ± s.e.m. average food consumption (µl) in consecutive 30-min segments during a 24-h LD cycle. **g**, Quantification (mean ± s.e.m. with individual data points) of daily total, daytime, nighttime and ZT0–4 sleep for flies after 7 days of group enrichment or social isolation. **h**, Quantification (mean ± s.e.m. with individual data points) of daily total, daytime, nighttime and ZT0–4 food consumption for flies after 7 days of group enrichment or social isolation. **e**–**h**, $n = 28$–30 flies; two-sided unpaired *t*-tests with Welch's correction; $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$. For *n* and *P* values, see Source Data.

analysis suggested that these 214 genes are enriched for biological pathways associated with oxidation–reduction processes ($P = 1.92 \times 10^{-18}$), one-carbon metabolic processes ($P = 1.38 \times 10^{-6}$) and carbohydrate metabolic processes ($P = 4.74 \times 10^{-6}$). The rest of the gene ontology of biological pathways showed a strong preference for metabolic functions, such as fatty acid, pyruvate, glucose and amino acid metabolic processes. Consistent with the sleep loss phenotype, sleep was also among the most overrepresented gene ontologies for biological pathways (Extended Data Fig. 5e, Supplementary Information).

Among the top 20 genes in this list, two genes stood out: *Limostatin* (*Lst*, CG8317), expression of which increased 1.67-fold after chronic isolation (Fig. 2d, ranked no. 6), and *Drosulfakinin* (*Dsk*), expression of which decreased 2.03-fold after chronic isolation (Fig. 2d, ranked no. 15). Limostatin is a decretin hormone that is induced by starvation and suppresses insulin release[13]. Drosulfakinin, a satiety-inducing cholecystokinin-like peptide, is secreted when the animal is fed[14]. As a signal of satiety, drosulfakinin is depleted under starvation conditions. A third gene, *target of brain insulin* (*tobi*), also showed significantly

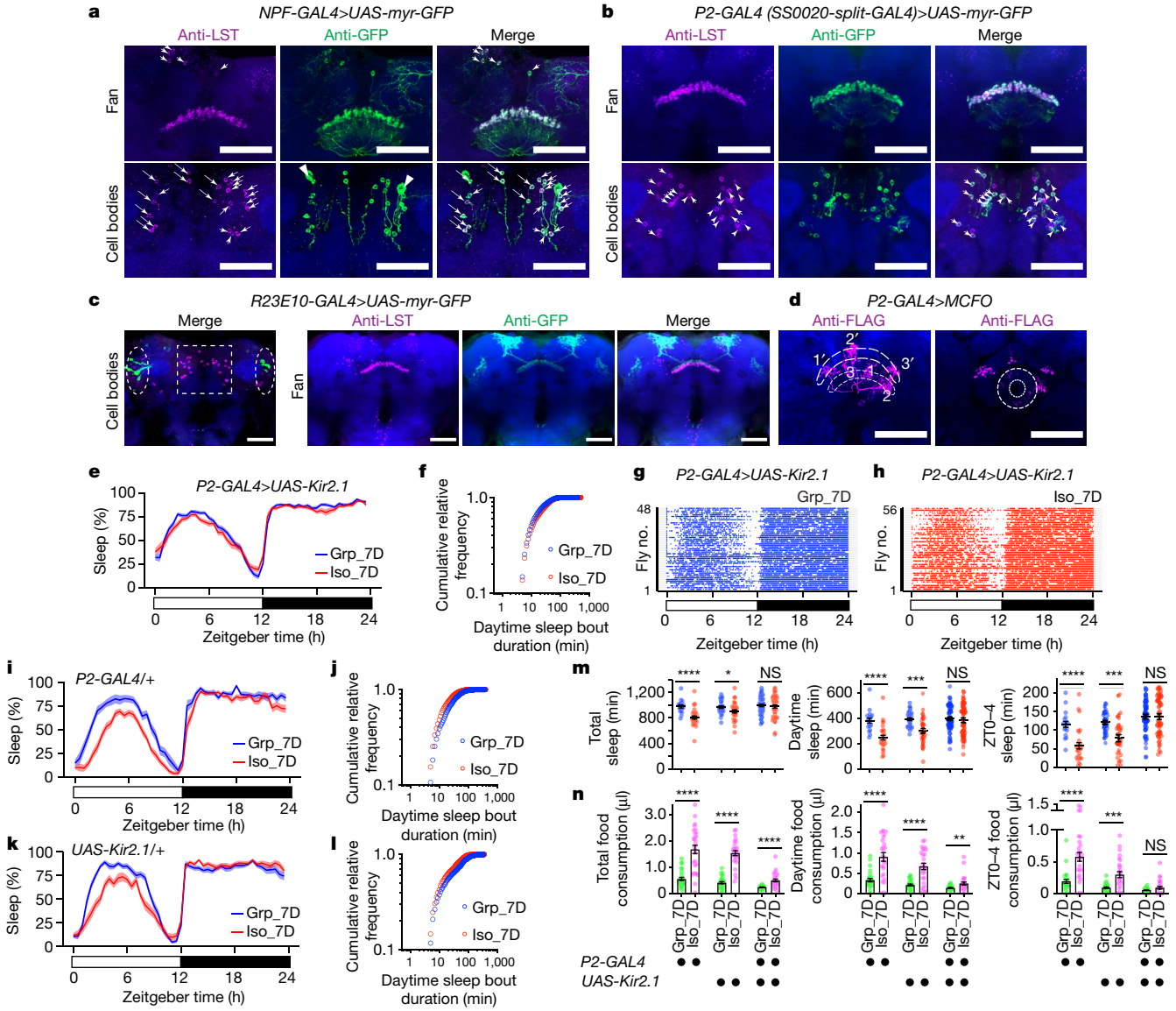

Fig. 3 | P2 neurons are required for sleep loss induced by chronic social isolation. a, LST-immunoreactivity-positive cells overlap *NPF-GAL4*-labelled fan-shaped body neurons at the fan layer and cell body levels. Arrows, LST-immunoreactivity-positive cell bodies (magenta) that are also GFP-positive (green). Arrowheads, NPF P1 neurons. **b**, LST-immunoreactivity-positive cells overlap *P2-GAL4(SS0020-split-GAL4)*-labelled NPF P2 neurons at the fan layer and cell body levels. Arrows, the majority of LST-immunoreactivity-positive cell bodies (magenta) are also GFP-positive (green), consistent with previous reports that *P2-GAL4* labels about 85% of NPF P2 neurons[22]. **c**, Expression pattern of *R23E10-GAL4* (GFP; ovals, cell bodies) and distribution of LST-immunoreactivity-positive cells (square, cell bodies). **a**–**d**, Blue, N-cadherin; scale bars, 50 µm. **d**, Left, fan-shaped body projections of three P2 neurons decorated by Flag tag (magenta). Each arborizes at a column of the lower layer of the FB (bottom dashed outline) and projects to a different column of the higher layer of the FB (top dashed outline; projections, 1→1′, 2→2′, 3→3′). Right, these three neurons also arborize at the ellipsoid body level (donut-shaped area). Blue, Bruchpilot; scale bars, 50 µm. **e**–**h**, Sleep profile (**e**), distribution of daytime sleep bout durations (**f**) and sleep bout raster plots (**g**, **h**) of flies expressing *UAS-Kir2.1* with *P2-GAL4* after group enrichment or social isolation for 7 days ($n = 48$–56 flies). **i**–**l**, Sleep profiles (**i**, **k**) and distributions of daytime sleep bout durations (**j**, **l**) of parental control flies after group enrichment or social isolation for 7 days ($n = 19$–32 flies). **m**, Quantification (mean ± s.e.m. with individual data points) of daily total, daytime and ZT0–4 sleep for all experimental and heterozygous control flies. **n**, Quantification (mean ± s.e.m. with individual data points) of daily total, daytime, and ZT0–4 food consumption for all experimental and heterozygous control flies ($n = 27$–30 flies). Sleep profiles, mean ± s.e.m. average proportion of time spent sleeping in consecutive 30-min segments during a 24-h LD cycle. **m**, **n**, Two-sided unpaired *t*-tests with Welch's correction; $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$; NS, not significant. For *n* and *P* values, see Source Data.

increased expression (1.76-fold) during chronic social isolation (Fig. 2d, ranked no. 4). *tobi* encodes an α-glucosidase that is regulated by *Drosophila* insulin and glucagon analogues[15]. In addition, 7 of these top 20 genes and 32 of the total 214 candidate genes were previously identified as being regulated in *Drosophila* heads after 24 h of starvation[16] (Fig. 2d, Supplementary Information). Thus, from a transcriptomic perspective, the brain of a fly maintained in chronic social isolation closely resembles the brain of a starving fly, despite continuous access to food. We reasoned that such a 'starvation brain state' might broadly affect gene expression associated with metabolic processes. Massive changes in mitochondrial functions and oxidation–reduction processes (Extended Data Fig. 5e) could be direct consequences of starvation and/or elevated feeding.

We used the activity recording capillary feeder (ARC) assay, a video recording capillary feeder (CAFE) assay that monitors sleep and feeding behaviours simultaneously and continuously in individual *Drosophila*[17].

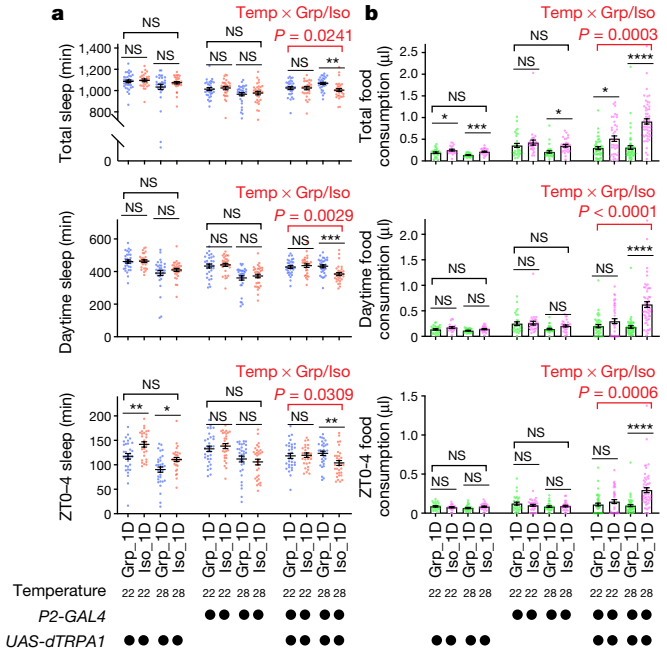

Fig. 4 | Activation of P2 neurons during acute social isolation induces sleep loss and over-consumption of food. For thermoactivation of P2 neurons, flies in group enrichment or social isolation were kept at 28 °C for 1 day. After 1 day of thermal activation (or no activation), sleep or feeding behaviour was measured at 22 °C. Flies kept at 22 °C (no thermoactivation) were used as controls. **a**, Quantification (mean ± s.e.m. with individual data points) of daily total, daytime and ZT0–4 sleep for experimental and heterozygous control flies grouped or isolated for 1 day with (28 °C) or without (22 °C) thermal activation of *P2-GAL4*-labelled neurons (n = 28–32 flies). **b**, Quantification (mean ± s.e.m. with individual data points) of daily total, daytime, and ZT0–4 food consumption for experimental and heterozygous control flies grouped or isolated for 7 days with (28 °C) or without (22 °C) thermal activation of *P2-GAL4*-labelled neurons (n = 22–57 animals). Two-way ANOVAs were used to detect interactions between temperature treatment and isolation status (Temp × Grp/Iso). Šidák multiple comparisons tests were used for post hoc comparisons between group-treated and isolated flies of the same genotype and temperature treatment. *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001; NS, not significant. For n and P values, see Source Data.

ARC assays validated the isolation-induced sleep loss phenotype previously observed with DAM assays: daily total sleep, daytime sleep and ZT0–4 sleep were reduced significantly after 7 days of social isolation. In addition, nighttime sleep was also reduced (Fig. 2e, g), probably owing to higher sensitivity in detecting movements using the positional tracking method, or differences in chamber shape and food source between the ARC and DAM systems. As predicted from the gene expression profiling results, we observed increased feeding in socially isolated animals compared to their group-treated counterparts (Fig. 2f, h). Flies isolated for 7 days showed significant increases in total food consumption, daytime food consumption, nighttime food consumption and ZT0–4 food consumption in comparison to flies that were group-housed for 7 days. Extended Data Fig. 6a, b shows sleep and feeding profiles in representative individual animals. Thus, chronic social isolation induces a starvation state in *Drosophila* at the levels of both gene expression in the brain and behaviour.

The altered feeding pattern produced by chronic social isolation is not merely a consequence of sleep loss, because several classic sleep mutants all exhibited normal feeding behaviour[18–20] (Extended Data Fig. 6c–e). In addition, acutely isolated flies did not show a strong increase in food consumption (Extended Data Fig. 6f, g).

Neurons for isolation-induced sleep loss

The candidate gene *Lst* is normally induced by nutrient restriction in endocrine neurons in the corpora cardiaca[13]. However, our RNA profiling experiment suggested that there could be a previously unknown brain source for LST production (Extended Data Fig. 7a, b, Supplementary Information). A resource of high-resolution transcriptomes of 100 GAL4 driver lines suggested that cells labelled by the driver line *NPF–GAL4* (NPF, neuropeptide F (the fly homologue of neuropeptide Y)) are likely to express LST[21]. Using a monoclonal antibody against LST[13], we detected co-localized LST immunoreactivity and *NPF–GAL4*-driven GFP signals (Fig. 3a). Among six known neuronal clusters that express NPF (Extended Data Fig. 7c, d), LST immunoreactivity appeared to be co-localized with *NPF–GAL4*-driven GFP signals at the dorsal stratum of the fan-shaped body (dorsal fan-shaped body, dFB) and in a cluster of small cell bodies in the dorsal brain (Fig. 3a). Neurons without known function that comprise this cluster of NPF cells were named P2 previously[22] (Extended Data Fig. 7c, d). A recent study used a split-GAL4 driver, *SS0020-split-GAL4* (abbreviated as *P2-GAL4* below), to strongly label the majority of P2 neurons that showed positive immunoreactivity for LST and NPF[22] (Fig. 3b).

Notably, the projections of the P2 neurons overlapped with the axonal projections of the dFB neurons labelled by *R23E10–GAL4*[23,24], which suggests that P2 neurons might signal to sleep-promoting dFB neurons. At the cell body level, P2 neurons differ from the *R23E10–GAL4* labelled cells (Fig. 3c). We used a MultiColor FlpOut (MCFO) approach[25] to stochastically decorate individual neurons labelled by *P2–GAL4*, and found that they are fan-shaped body columnar neurons (Fig. 3d, Supplementary Information). The hemibrain connectome[26] allowed us to determine that P2 neurons include, as a dominant constituent, the hDeltaK cell type—a columnar cell class, where each neuron has a stereotypical dendritic input in the ellipsoid body (EB) in addition to the FB innervation[26,27] (Fig. 3d and Supplementary Information). hDeltaK cells exhibit extensive synaptic connections with a known subset of *R23E10–GAL4*-labelled sleep-promoting dFB neurons[27] (Extended Data Fig. 8, Supplementary Information). On the basis of the above connectome data and existing evidence that NPF/NPY is involved in animal metabolism and stress responses, we focused on P2 neurons.

To test whether P2 neurons contribute to sleep loss induced by chronic social isolation, we chronically silenced these neurons by expressing the inward-rectifying potassium channel Kir2.1[28] under the control of *P2–GAL4*. In flies carrying both *P2–GAL4* and *UAS–Kir2.1*, chronic isolation no longer induced an altered sleep profile when compared to their group-housed counterparts (Fig. 3e). The cumulative relative frequency curve of daytime sleep bouts for socially isolated animals no longer climbed faster than that of group-reared animals (Fig. 3f). Raster plots of sleep bouts in individual flies showed little difference between chronically isolated and group-housed flies (Fig. 3g, h). We found no difference between isolated and group-housed animals for daily total sleep, daytime sleep, or ZT0–4 sleep (Fig. 3m). By contrast, in heterozygous parental control animals carrying either the *P2–GAL4* or the *UAS–Kir2.1* transgene, chronic social isolation robustly induced sleep loss (Fig. 3i–m). Temporally silencing P2 neurons using *UAS–shibire^{ts1}* during group enrichment or social isolation did not block social isolation-induced sleep loss (Extended Data Fig. 7 h–n, Supplementary Information). Although isolated flies carrying both *P2–GAL4* and *UAS–Kir2.1* still showed some overconsumption of food, they no longer showed excessive food consumption for ZT0–4 (Fig. 3n), and the total increase in daytime food consumption was much smaller than in parental controls (Fig. 3n, Extended Data Fig. 7e–g).

Misperceiving social isolation duration

Using [Ca^{2+}] imaging, we found that the activity of P2 neurons was correlated with locomotor activity in both group-housed and isolated flies

(Extended Data Fig. 8). One might expect that P2 neurons would be tonically more active in isolated flies than in group-housed flies, but we could not detect this effect in baseline [Ca^{2+}] levels (Extended Data Fig. 8). Alternatively, we can hypothesize that locomotion drives more P2 neuron total activity during 7 days of isolation than during 1 day of isolation.

We therefore tested whether boosting activity in P2 neurons during acute social isolation (1 day) is sufficient to promote behavioural changes that resemble the effects of chronic social isolation (7 days). To activate P2 neurons, we expressed a *Drosophila* warmth-gated cation channel, *UAS–dTRPA1*, with *P2-GAL4*. The *P2–GAL4*-labelled neurons were activated by treating the flies at 28 °C during acute social isolation or group enrichment (1 day) (Extended Data Fig. 9a, for experiments in which P2 neurons were activated for 7 days, see Extended Data Fig. 10 and Supplementary Information). Control experiments, using flies of the same genotype, were conducted by treating the flies at 22 °C during acute social isolation or group enrichment (1 day). Following these treatments, all flies were subsequently maintained at 22 °C for measurement of sleep or feeding behaviour (Fig. 4). In animals carrying both *P2–GAL4* and *UAS–dTRPA1*, there were significant interactions between temperature treatment and isolation status for total, daytime, and ZT0–4 sleep and food consumption: activation of P2 neurons during acute social isolation promoted significant sleep loss and excessive feeding (Fig. 4, Extended Data Fig. 9), whereas activation of P2 neurons in group housing did not alter either sleep or feeding behaviour. In control experiments, we found no evidence for interactions between temperature treatment and isolation status in the heterozygous parental flies (Fig. 4, Extended Data Fig. 9).

Discussion

Notably, P2 neurons are connected to the dFB neurons that are known to regulate sleep homeostasis and couple energy metabolism to sleep[23,29–31]. Artificial activation of P2 neurons can produce a behavioural state that resembles the effects of chronic isolation after social isolation for a single day; however, activation of P2 neurons failed to produce these behaviours in the complete absence of social isolation (Fig. 4, Extended Data Fig. 9). This indicates that both activity in P2 neurons and a status of being socially isolated are required to induce reduced sleep and increased feeding. Social isolation might be sensed by P2 neurons or elsewhere in the brain, but in either case appears to cause the activity of P2 neurons to be interpreted differently and thereby to generate novel behaviours. Downregulation of a secreted cytokine in a non-neural tissue—the fat body—suppresses sleep and promotes feeding in *Drosophila*[32]. It would be interesting to determine whether these behavioural responses also depend on P2 neuronal activity.

Modifications of feeding circuits appear to be crucial for the evolution of complex social behaviours. For example, in *C. elegans*, a single-residue difference in the neuropeptide Y receptors of naturally occurring strains determines whether the strains exhibit solitary or social feeding behaviour[33]. As antibodies to neuropeptide F, the fly homologue of neuropeptide Y, label P2 neurons, we hope to ascertain in future work whether *Drosophila*'s P2 neurons influence social patterns of feeding behaviour as well as mediating feeding and sleep responses to social isolation.

In humans, social isolation promotes new emotional states that intensify with the passage of time. We have found that sleep loss in *Drosophila* is a faithful readout of the duration of social isolation, and this allowed us to identify specific patterns of gene and behavioural states that emerge as social isolation becomes chronic. This unexpected association between social isolation, sleep and metabolism in an insect model is reminiscent of the connection observed by social psychologists between loneliness, sleep difficulties and hyperphagia. Such robust findings in *Drosophila* suggest that studies of animal models might identify conserved brain states, genes, and neural circuits that are associated with social isolation.

Online content

Any methods, additional references, Nature Research reporting summaries, source data, extended data, supplementary information, acknowledgements, peer review information; details of author contributions and competing interests; and statements of data and code availability are available at https://doi.org/10.1038/s41586-021-03837-0.

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Methods

No statistical methods were used to predetermine sample size. Control and experimental flies were reared in identical conditions, and were randomized whenever possible (location in housing incubator,animal selection, position in behavior monitor systems, and so on). Group-housed and isolated animals of the same genotype or condition were always tested in parallel (for example, same batch of fly food, same incubator and same testing period). Data analyzer (software) was blinded when assessing the result.

Drosophila strains and culture

D. melanogaster stocks were raised on standard medium (cornmeal/yeast/molasses/agar) at 22 °C under 12-h light: 12-h dark (LD) cycles. The wild-type isogenic strain *Canton-S* w^{1118} *(iso1CJ)*[34] was used as controls for behaviour and RNA-seq experiments. Additional isogenic strains, including *Canton-S* and *Berlin-K*, were used for testing the effects of group housing or isolation on sleep. *Canton-S* and *UAS-GCaMP7f* flies were provided by G. Maimon and *Berlin-K* flies were obtained from the Bloomington *Drosophila* Stock Center. Sleep inbred panel (SIP) lines (SIP-L1-3, SIP-L1-4, SIP-L2-1,SIP-L2-6, SIP-S1-1, SIP-S1-9, SIP-S2-3 and SIPS2-9)[35,36] were obtained from the Bloomington *Drosophila* Stock Center. The sleep mutant strain *insomniac*^{1} (ref. [20]) was a standing stock of the laboratory. The sleep mutant *fumin*[18] was provided by A. Sehgal. The sleep mutant *wake*^{D2} (ref. [19]) was provided by M. Wu. All sleep mutants tested in this study were backcrossed to the wild type: *Canton-S* w^{1118} *(iso1CJ)* for at least five generations. *NPF-GAL4*, *SS0020-split-GAL4* (*P2-GAL4*)[22] and *UAS-Kir2.1* (*pJFRC49-10XUAS-IVS-eGFPKir2.1*) flies were gifts from L. Shao and U. Heberlein. *UAS-myr::GFP* (*attP40*) (*pJFRC12-10XUAS-IVS-myr::GFP*), *GMR23E10-GAL4* (*attP2*), *UAS-dTRPA1* (*attP16*), *MCFO-3* (*GMR57C10-FlpL(su(Hw)attP8);; 10xUAS(FRT.stop) myr::smGdP-HA(VK00005)*, *10xUAS(FRT.stop)myr::smGdP-V5-THS-10xUAS(FRT.stop)myr::smGdP-FLAG* (*su*(*Hw*)*attP1*)[25], and *UAS-Shibire*^{ts1} (*VK00005*) (*pJFRC100-20xUAS-TTS-Shibire-ts1-p10*) flies were obtained from the Bloomington *Drosophila* Stock Center. All experiments were conducted in male animals. Female animals also exhibit the chronic social isolation-induced sleep loss phenotype but were not used in this study.

Group enrichment and social isolation

Newly eclosed flies were collected and kept in standard fly food bottles (~200 flies per bottle) for 3–5 days to acquire social experience. Mating was allowed to happen freely during this period. Male flies were then sorted into standard fly food vials: 1 fly per vial for social isolation (Iso) and 25 flies per vial for group enrichment (Grp). For Extended Data Figs. 1, 3f, g, we varied group sizes for group enrichment: 2 flies per vial for Grp(2), 5 flies per vial for Grp(5), 25 flies per vial for Grp(25), 100 flies per vial for Grp(100). For Extended Data Fig. 3m, n, we varied the sex composition of the group: 30 males were housed per vial (male-only group) or 15 males and 15 females (mixed-sex group). For all experiments, the fly sorting day was considered day 0. On day 1, day 3, day 5 or day 7, isolated or group-housed flies were used for sleep measurements, feeding measurements or RNA-seq experiments.

Sleep measurement and analysis

Locomotor activity of flies was monitored using the DAM system (TriKinetics, Waltham, MA). Flies with group or isolation experience were loaded into glass tubes containing fly culture food and assayed at 22 °C under 12-h LD cycles. Activity counts were collected at 1-min bins for three LD cycles after the loading day. Sleep parameters, sleep profiles and sleep bout distributions were analysed based on activity counts using the R3.6/Rethomics package[37]. For sleep profile and sleep parameters presented here, data from three LD cycles of the same animal were averaged to generate sleep profile, daily total sleep, daytime sleep, nighttime sleep, and ZT0–4 sleep.

Sleep bout distribution analysis

After calculating the duration of every sleep bout for animals measured for three LD cycles immediately after group or isolation treatment, all daytime (or nighttime) sleep bouts from all animals tested for a given condition (certain genotype, group or isolated after certain number of days) were pooled together. The pooled data were used to generate density plots and cumulative relative fraction plots. For density plots, a bin size of 10 min was used and the plot was generated using the geom_histogram function of the R/ggplot2_3.3.3 package. For cumulative relative fraction plots, sleep episode duration was treated as a continuous variable with a bin size of 1 min. The cumulative relative fraction for any given sleep episode duration (min) was calculated as the total number of bouts equal to and shorter than that sleep episode duration divided by the total number of all bouts (daytime or nighttime). The resulting cumulative relative fraction was plotted on the *y*-axis and the *x*-axis represented the continuous time interval with a bin size of 1 min.

RNA purification

Heads of wild-type flies with group or isolation experience were collected during ZT0.5–2. Flies of three conditions were collected: socially enriched animals (group enrichment for 7 days, Grp), chronically isolated animals (isolated for 7 days, Iso_7D) and acutely isolated animals (isolated for 1 day, Iso_1D). For each condition, three replicated samples were collected, and each sample contained 200 fly heads. Total RNA was extracted using TRIzol reagents and homogenized using a BeadBug microtube homogenizer (Benchmark Scientific). Samples were further extracted using chloroform and the aqueous phase containing nucleic acid was acquired with the assistance of Phase Lock Gel, Heavy (QuantaBio). We then used the RNeasy Mini Kit (Qiagen) to remove DNA with DNase and further purify the samples, following the manufacturer's protocol.

RNA-seq and differential gene expression analysis

RNA-seq was conducted at the Genomic Resources Center of the Rockefeller University. Sequencing libraries were prepared with the Illumina TruSeq stranded mRNA LT kit. We used 100 ng total RNA for each sample. Libraries were multiplexed and sequenced on the Illumina NextSeq 500 sequencer using high output V2 reagents and NextSeq Control Software v1.4 to generate 75-bp single reads, according to the manufacturer's protocol. The sequencing depth was at least 50 million reads per sample. Reads were aligned to the FlyBase release 6.13 (May 2016) genome assembly with STAR_2.4.2a[38] and read counts were generated using featureCounts_1.5.0[39]. Differential expression analysis was performed with DESeq2_1.26.0[40]. Gene ontology (GO) analysis was performed using the Database for Annotation, Visualization and Integrated Discovery (DAVID_6.8)[41].

ARC assay

To measure feeding behaviour in individual animals, we used an ARC assay (detailed protocol as described[17]). In brief, in the ARC assay, flies were housed in customized chambers with 1% agar and fed on liquid food (2.5% sucrose and 2.5% yeast) from microcapillaries. Food consumption was measured by video tracking the liquid meniscus (made from Dodecane, a copper dye, and mineral oil) over time, while sleep measurements were obtained from positional tracking of the same animal in the chamber. Frames were acquired at 1 Hz using Open CV_3.2.0 (The MacPorts Project). Object tracking was performed using JavaGrinders (Oracle). ARC system data were analysed using the Python_3.5/Noah_15.8 script. In each experiment, two ARC chambers were recorded simultaneously, and the flies were loaded into the two chambers in a genotype/treatment-balanced manner, so biases from devices were minimized. We loaded flies immediately after group enrichment or social isolation around ZT8–9 and sleep/feeding analyses started at

ZT12 on the loading day and ended after ZT36. Sleep or feeding data were binned for every 30 min for display of sleep or feeding profiles. ZT24–ZT36 was presented as ZT0–12 in figures to display the results of a full LD cycle. Dead flies, flies that failed to consume any liquid food from the microcapillaries and flies housed in chambers with tracking errors were excluded from the analyses.

Immunohistochemistry and confocal microscopy

Flies were briefly anaesthetized with CO_2 and dissected in cold Schneider's *Drosophila* medium. Dissected brains were fixed in 2% paraformaldehyde in Schneider's *Drosophila* medium at room temperature (RT) for 1 h in a vacuum chamber. The brains were washed four times for 15 min each in PBT (phosphate-buffered saline (PBS) with 0.5% Triton X-100) at room temperature, blocked for 2 h at room temperature with blocking buffer (PBT + 5% normal goat serum) and incubated with primary antibodies in blocking buffer, overnight on a nutator at 4 °C. The primary antibodies and their dilutions used were: rabbit anti-GFP (1:500, Thermo Fisher Scientific, A6455), mouse anti-GFP (1:500, Thermo Fisher Scientific, A11120), mouse anti-LST (1:1,000, gift from Dr Seung Kim, Stanford University), rabbit anti-NPF (1:500, RayBiotech, RB-19-0001), rat anti-N-cadherin (1:200, Developmental Studies Hybridoma Bank, DN-Ex #8), rat anti-Flag-tag (1:250, Novus, NBP1-06712SS), rabbit anti-HA-tag (1:250, Cell Signaling Technology, C29F4), and mouse anti-Bruchpilot (1:50, Developmental Studies Hybridoma Bank, nc82). This was followed by four washes for 20 min each in PBT at room temperature, and incubation overnight on a nutator at 4 °C with secondary antibodies in blocking buffer. The secondary antibodies were: Alexa Fluor 488 anti-rabbit (1:500, Jackson ImmunoResearch, 111-545-144), Alexa Fluor 488 anti-mouse (1:500, Thermo Fisher Scientific, A11029), Alexa Fluor 568 anti-mouse (1:500, Thermo Fisher Scientific, A11031), Alexa Fluor 568 anti-rat (1:500, Thermo Fisher Scientific, A11077), Alexa Fluor 647 anti-rat (1:500, Jackson Immunoresearch, 112-605-167) and Alexa Fluor Plus 647 anti-rabbit (1:1,000, Thermo Fisher Scientific, A32733). Samples were then washed four times for 15 min each in PBT at room temperature and once for 15 min in PBS, mounted with Fluoromount-G mounting medium (Southern Biotech, 0100-01) and cured overnight at 4 °C. Samples were imaged on a Zeiss LSM710 confocal microscope. Images were processed with Zeiss ZEN 2.3 SP1 software and Fiji (ImageJ2) software.

Thermogenetic neuronal activation during social isolation

For experiments in Fig. 4, Extended Data Figs. 9, 10, fly crosses were reared at 22 °C under 12-h LD cycles. Progenies with the expected genotypes were collected and kept at 22 °C for 5 days to acquire social experience before being sorted for group enrichment or social isolation. Grouped or isolated flies were incubated at either 22 °C or 28 °C for either 1 day or 7 days. If the progenies expressed the *UAS-dTRPA1* transgene in *P2-GAL4*-labelled neurons, these neurons were thermogenetically activated at 28 °C during the 1-day or 7-day period of group enrichment or social isolation. The sleep of all animals was tested at 22 °C. *P2-GAL4*-labelled neurons were not thermogenetically activated during the behaviour test phase, and therefore activation of these neurons was restricted to the group enrichment or social isolation phase of 1 day or 7 days.

$[Ca^{2+}]$ imaging

Flies were tethered to a custom plate for imaging and walking on an air-cushioned ball in the dark. Dissection and imaging protocols have been previously described[42]. Data were collected using PrairieView 5.4 (Bruker). We used male flies carrying both *UAS-GCaMP7f* and *P2-GAL4* following 7 days of group enrichment or social isolation, and the imaging experiments were conducted at ZT0–4. Each fly was imaged for a duration of 5 min. We used the same acquisition parameters across all the flies with laser intensity at the back aperture at 30–40 mW. Flies from different groups were tethered and imaged alternatively to minimize potential circadian effects. We defined regions of interest (ROIs) for the lower layer and higher layer of P2 neurons separately and used these ROIs as the unit for calculating fluorescence intensities. For each ROI, we calculated the mean signal value across pixels at each time point. In Extended Data Fig. 8e, we report the raw, amplified photomultiplier tube signal. In Extended Data Fig. 8c, d, we normalized the raw fluorescence values using the $\Delta F/F_0$ method, where F_0 was defined as the mean of the lowest 5% of raw fluorescence values in a given ROI over time and ΔF was defined as $F - F_0$. Cross-correlation analyses were used to determine the relationship between neural activity and the fly's locomotion. To compare the activity level of P2 neurons between flies from the group enrichment and social isolation groups, we averaged the raw, amplified photomultiplier tube signal across moments when flies were standing, as the activity of P2 neurons is correlated with the fly's locomotion (Extended Data Fig. 8c, d). For a fly to be detected as standing, the translational speed needed to be less than 2 mm/s and the turning speed less than 50°/s.

Statistics and reproducibility

Statistical analyses for behavioural experiments were performed using GraphPad Prism 8. For comparisons between group-housed and isolated animals, two-tailed unpaired *t*-tests with Welch's correction were used. For comparisons between multiple groups, ordinary one-way ANOVAs followed by Tukey's multiple comparison tests were used. For comparing sleep bout distributions, Kolmogorov–Smirnov tests were used. For determining whether P2 neuron activation influences social isolation induced behaviour change, two-way ANOVAs were used for detecting interactions between temperature treatment and group/isolation status and Šidák multiple comparisons tests were used for post hoc analyses between group-treated and isolated animals of the same genotype and temperature treatment. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$. All behaviour experiments were repeated at a different time with different batch of flies to ensure reliable conclusions. Representative confocal images are shown from at least 10 independent samples examined in each case.

Reporting summary

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

Data availability

The RNA-seq data sets generated in this study have been deposited in NCBI's Gene Expression Omnibus[43] and are accessible through GEO series accession number GSE137498. Source data are provided with this paper.

Code availability

Customized R script based on the R/rethomics package is available upon request.

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Acknowledgements We thank G. Maimon for advice on [Ca^{2+}] imaging experiments and quantitative analysis of the hemibrain connectome; J. Park, W. Ja, L. Shao, U. Heberlein, S. Park, S. Kim, A. Sehgal, M. Wu and H. Steller for sharing fly stocks, reagents, protocols, and equipment; B. McEwen, L. Vosshall, A. Patke, L. Zhao, N. Svetec, Y. Shuai, S. Axelrod and D. Top for comments on the manuscript; the Resource Center of Precision Instrumentation Technologies and the Resource Center of Genomics at the Rockefeller University for technical support; the Bloomington *Drosophila* Stock Center for fly stocks; and the Developmental Studies Hybridoma Bank for antibodies. This work was supported by NIH grants 5R37 NS053087 and 5R35 GM136237 to M.W.Y. W.L. was supported by fellowships from the Leon Levy Foundation, the Jane Coffin Childs Memorial Fund, and the Grass Foundation. S.S. was partially supported by NSF IOS no. 1656603. C.L. was supported by a seed grant from the Kavli Foundation.

Author contributions W.L. conceived the project, designed experiments, analysed data, and wrote the manuscript. W.L., Z.W., S.L., J.O., A.D.N. and I.F. performed experiments. C.L. performed [Ca^{2+}] imaging experiments and analysed the data. S.S. supervised statistical analyses. M.W.Y supervised the project and wrote the manuscript. All authors discussed the results and contributed to the manuscript.

Competing interests The authors declare no competing interests.

Additional information

Supplementary information The online version contains supplementary material available at https://doi.org/10.1038/s41586-021-03837-0.

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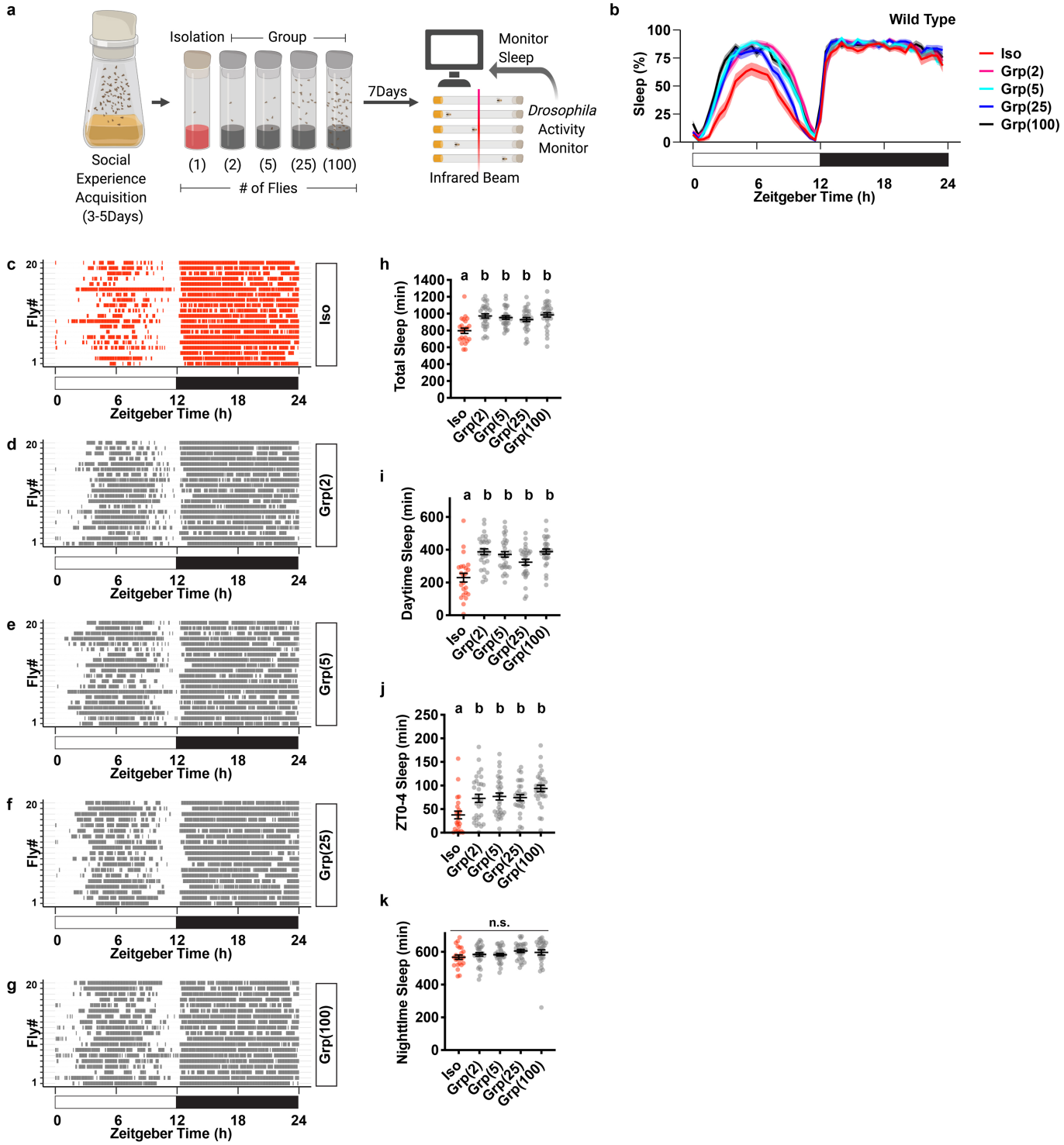
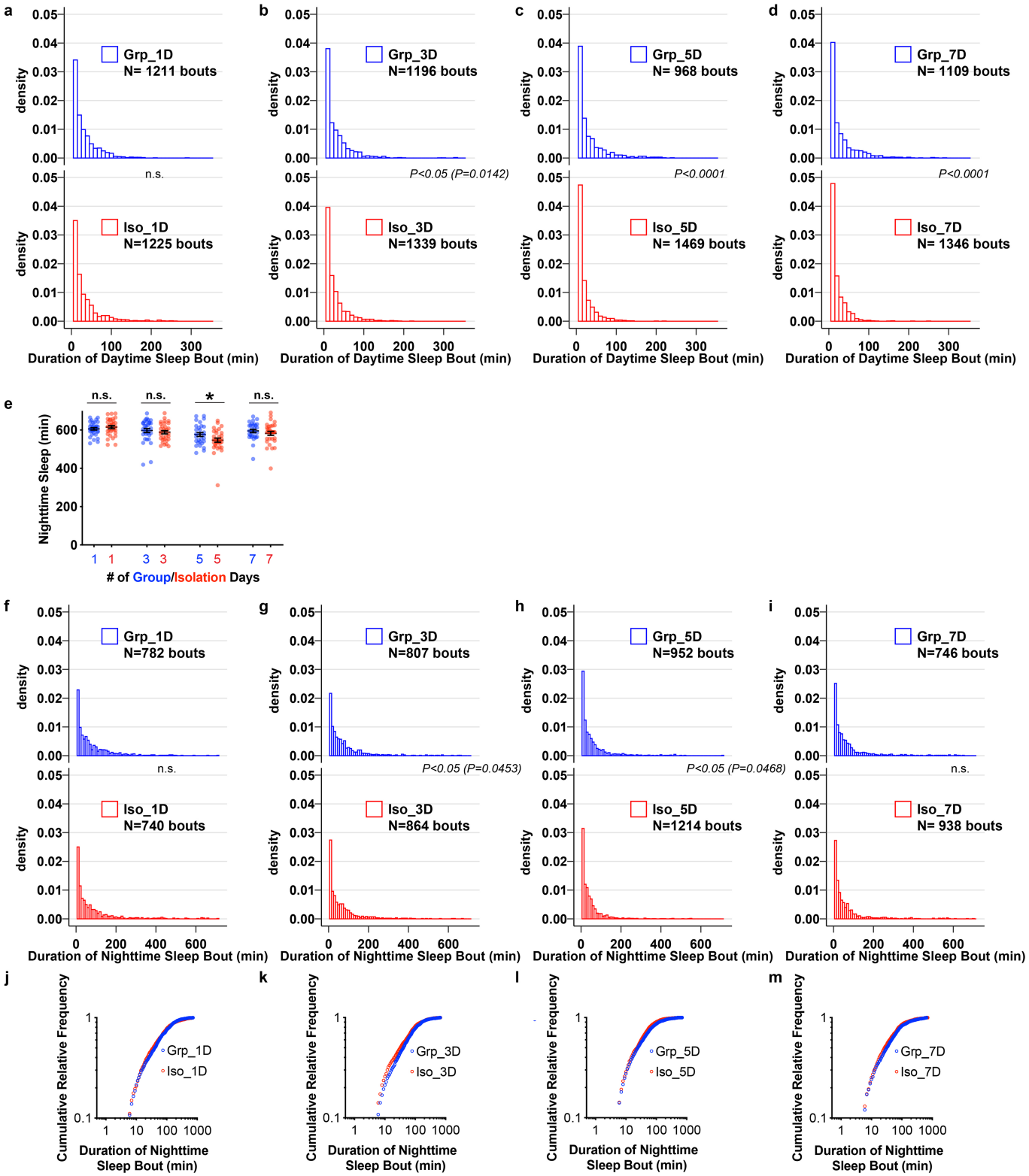
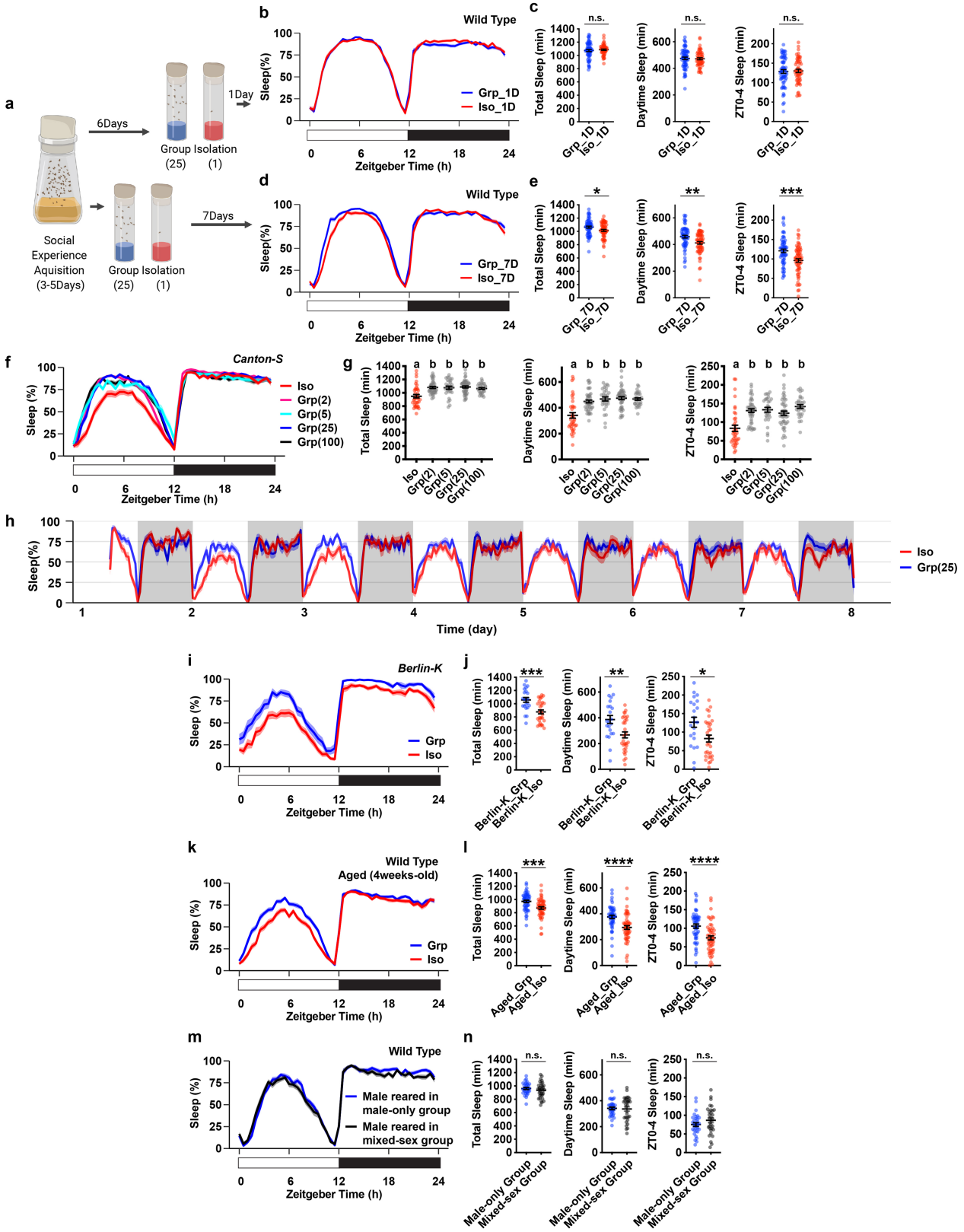
Peer review information *Nature* thanks Jason Rihel and the other, anonymous, reviewer(s) for their contribution to the peer review of this work.



Extended Data Fig. 1 | Social isolation reduces sleep in *Drosophila*. **a**, Schematic of social isolation paradigm. Adult fruit flies with social experience were subjected to social isolation or group enrichment for 7 days before sleep was measured using *Drosophila* activity monitors. Social isolation consists of housing one fly per vial. Group enrichment consists of housing 2, 5, 25 or 100 flies per vial. **b**, Sleep profiles (mean ± s.e.m. proportion of time spent sleeping in consecutive 30-min segments during a 24-h LD cycle) of flies after social isolation or group enrichment with different group sizes for 7 days. **c**–**g**, Raster plots of sleep bouts of 20 individual animals after social isolation (**c**), group enrichment in a group of 2 animals (**d**), group enrichment in a group of 5 animals (**e**), group enrichment in a group of 25 animals (**f**) or group enrichment in a group of 100 animals (**g**). Each row is an individual fly, with each coloured bar representing a sleep bout in a 24-h LD cycle. **h**–**k**, Quantification (mean ± s.e.m. with individual data points) of daily total sleep (**h**), daytime sleep (**i**), ZT0–4 sleep (**j**) and nighttime sleep (**k**) for flies after social isolation (Iso) or group enrichment (Grp) with different group sizes. For **b** and **h**–**k**, $n = 23$–30 flies. Ordinary one-way ANOVA followed by Tukey's multiple comparison tests; means sharing the same letter are not significantly different. For n and P values, see Source Data.

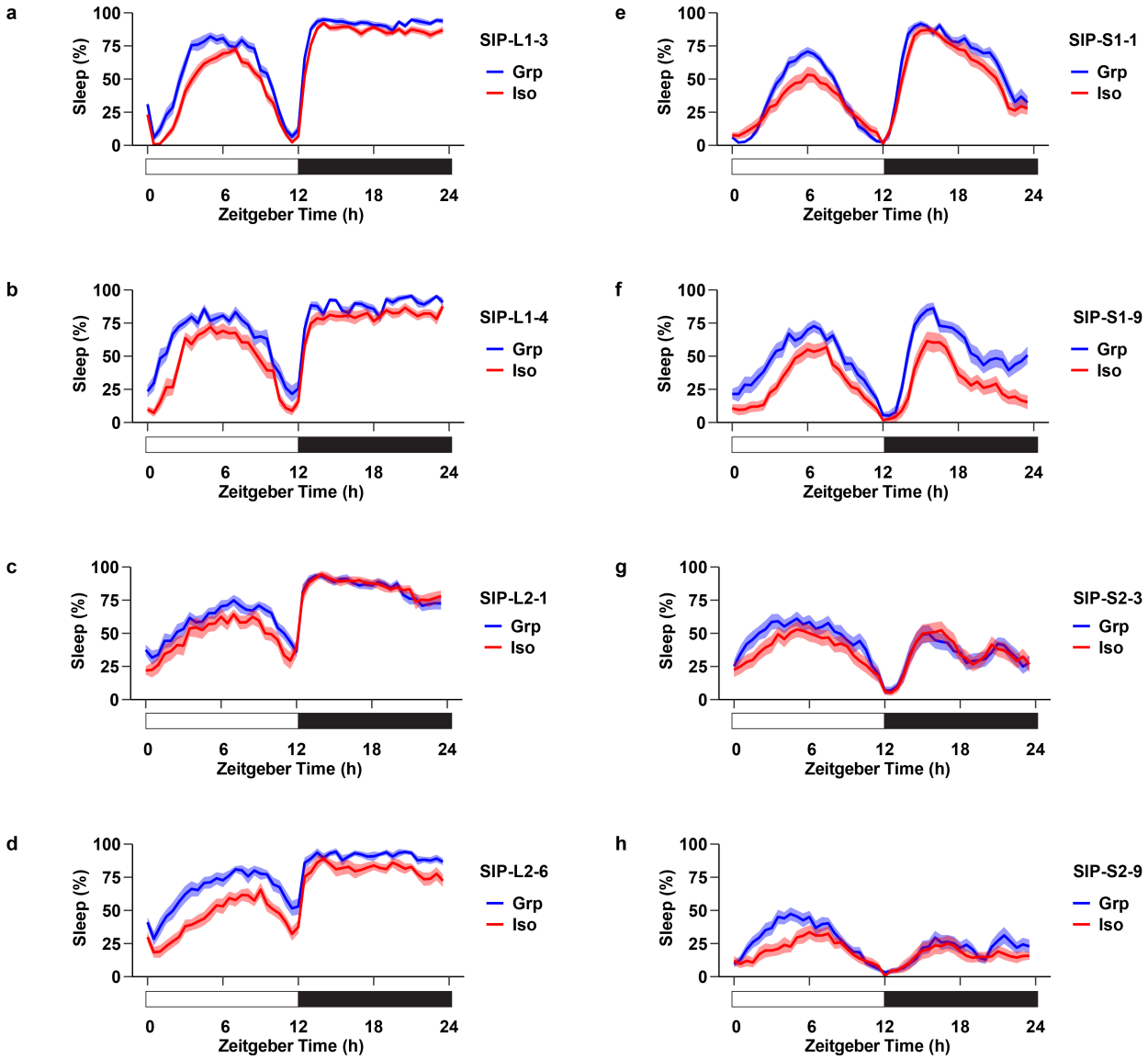


Extended Data Fig. 2 | Chronic social isolation does not alter nighttime sleep in wild-type *Drosophila*. **a**–**d**, Density plots for distribution of daytime sleep bouts for flies after 1, 3, 5 or 7 days of group enrichment or social isolation. All daytime sleep bouts collected from all animals in each condition were combined. **e**, Quantification (mean ± s.e.m. with individual data points) of daily nighttime sleep for wild-type flies after group enrichment or social isolation for 7 days. **f**–**i**, Density plots for distribution of nighttime sleep bouts for flies after 1, 3, 5 or 7 days of group enrichment or social isolation. All nighttime sleep bouts collected from all animals in each condition were combined. **j**–**m**, Plots of cumulative relative frequency for distributions of nighttime sleep bouts for flies after 1, 3, 5 or 7 days of group enrichment or social isolation. Kolmogorov–Smirnov tests were used to compare distributions. For **e**, $n = 29$–32 animals, two-sided unpaired t-test with Welch's correction; $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$. n.s., not significant. For n and P values, see Source Data.

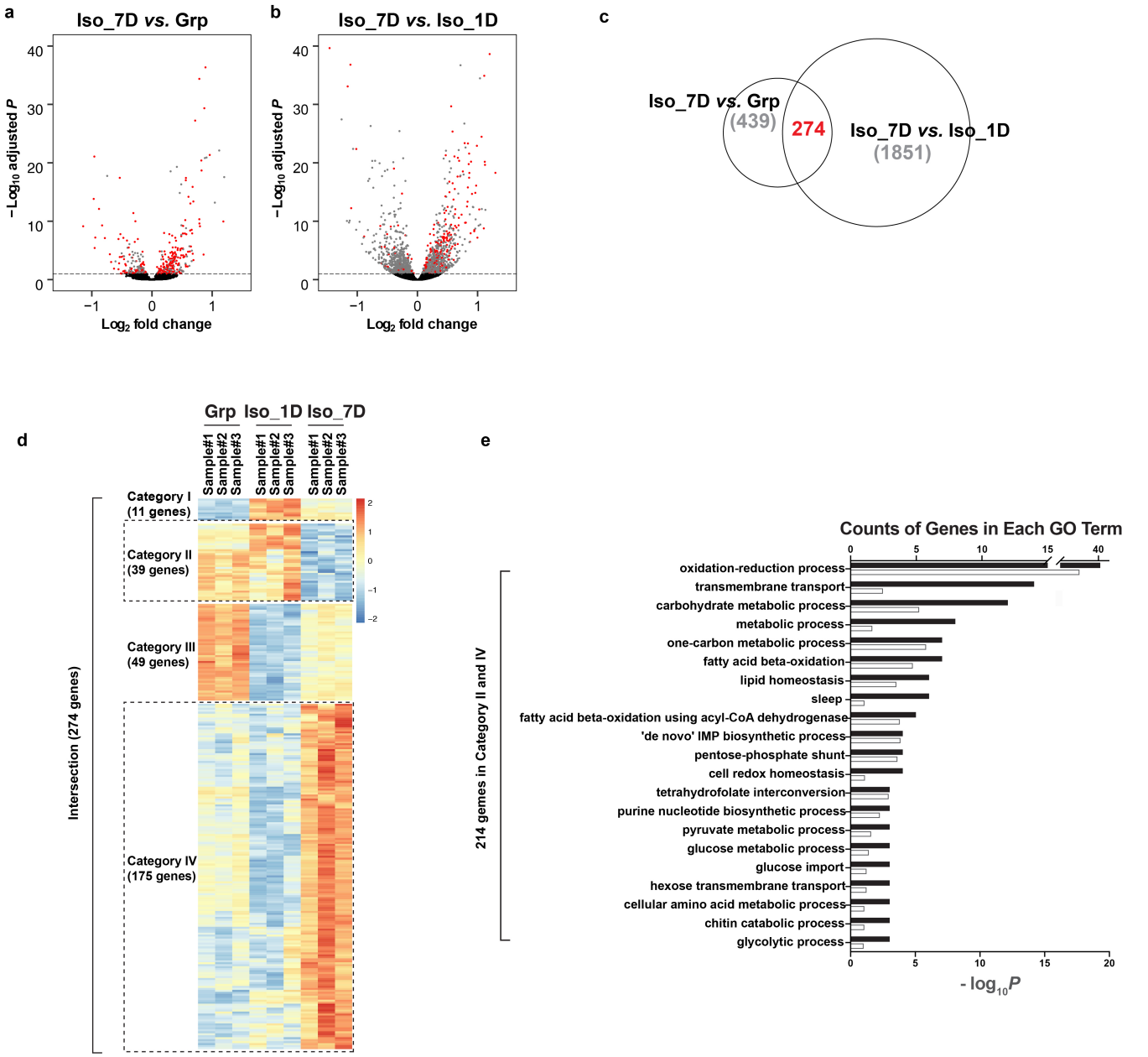


Extended Data Fig. 3 | See next page for caption.

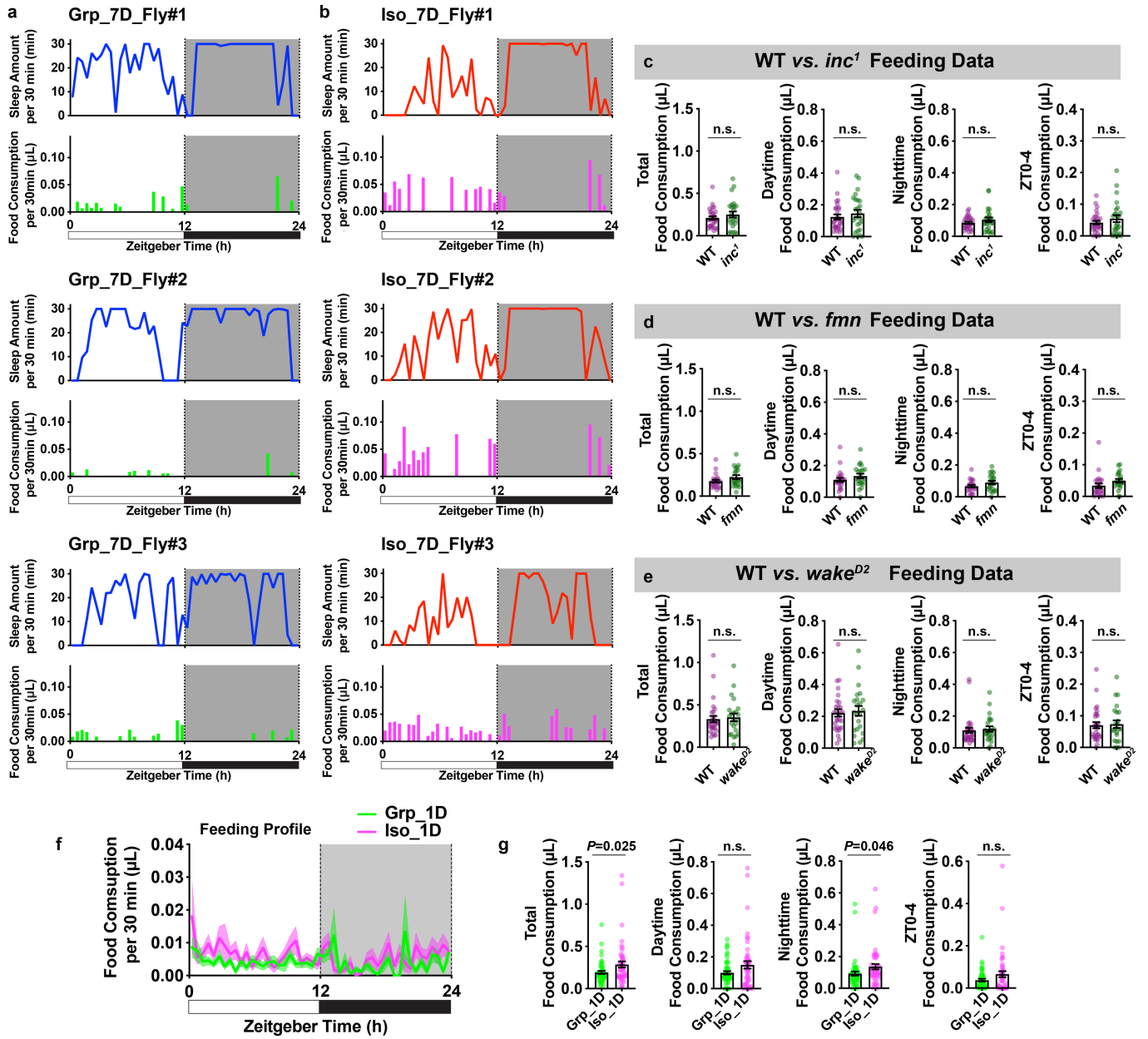
Extended Data Fig. 3 | Social isolation reduces *Drosophila* sleep in age-matched flies, in various isogenic strains, and in aged wild-type animals. **a**, Schematics of measuring sleep using *Drosophila* activity monitors after 1 or 7 days of group enrichment or social isolation in age-matched flies. **b**–**e**, Sleep profile and quantification of daily total sleep, daytime sleep and ZT0–4 sleep after 1 day (**b**, **c**, $n = 55–64$ flies) or 7 days of group enrichment or social isolation (**d**, **e**, $n = 61–64$ flies). **f**, **g**, Sleep profile and quantification of daily total sleep, daytime sleep and ZT0–4 sleep of the *Canton-S* isogenic strain after social isolation or group enrichment with different group sizes for 7 days ($n = 30–47$ flies). **h**, A 7-day-long sleep profile of flies after group enrichment or social isolation for 7 days. **i**, **j**, Sleep profile and quantification of daily total sleep, daytime sleep and ZT0-4 sleep for *Berlin-K* flies after social isolation or group enrichment (25 flies in a group) for 7 days ($n = 22–31$ flies). **k**, **l**, Sleep profile and quantification of daily total sleep, daytime sleep and ZT0–4 sleep of aged wild-type flies after group enrichment or social isolation (25 flies in a group) for 7 days ($n = 52–54$ animals). **m**, **n**, Sleep profile and quantification of daily total sleep, daytime sleep and ZT0–4 sleep of male wild-type flies after group enrichment in a male-only group (30 flies in a group) or in a mixed-sex group (15 male and 15 female flies in a group) for 7 days ($n = 32$ animals). Sleep profiles are displayed as the average proportion of time spent sleeping in consecutive 30-min segments during a 24-h LD cycle (mean ± s.e.m.). Quantifications are displayed as mean ± s.e.m. with individual data points. For **g**, ordinary one-way ANOVA followed by Tukey's multiple comparison test; means sharing the same letter are not significantly different. For **c**, **e**, **j**, **l** and **n**, two-sided unpaired *t*-tests with Welch's correction; $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$; n.s., not significant. For *n* and *P* values, see Source Data.



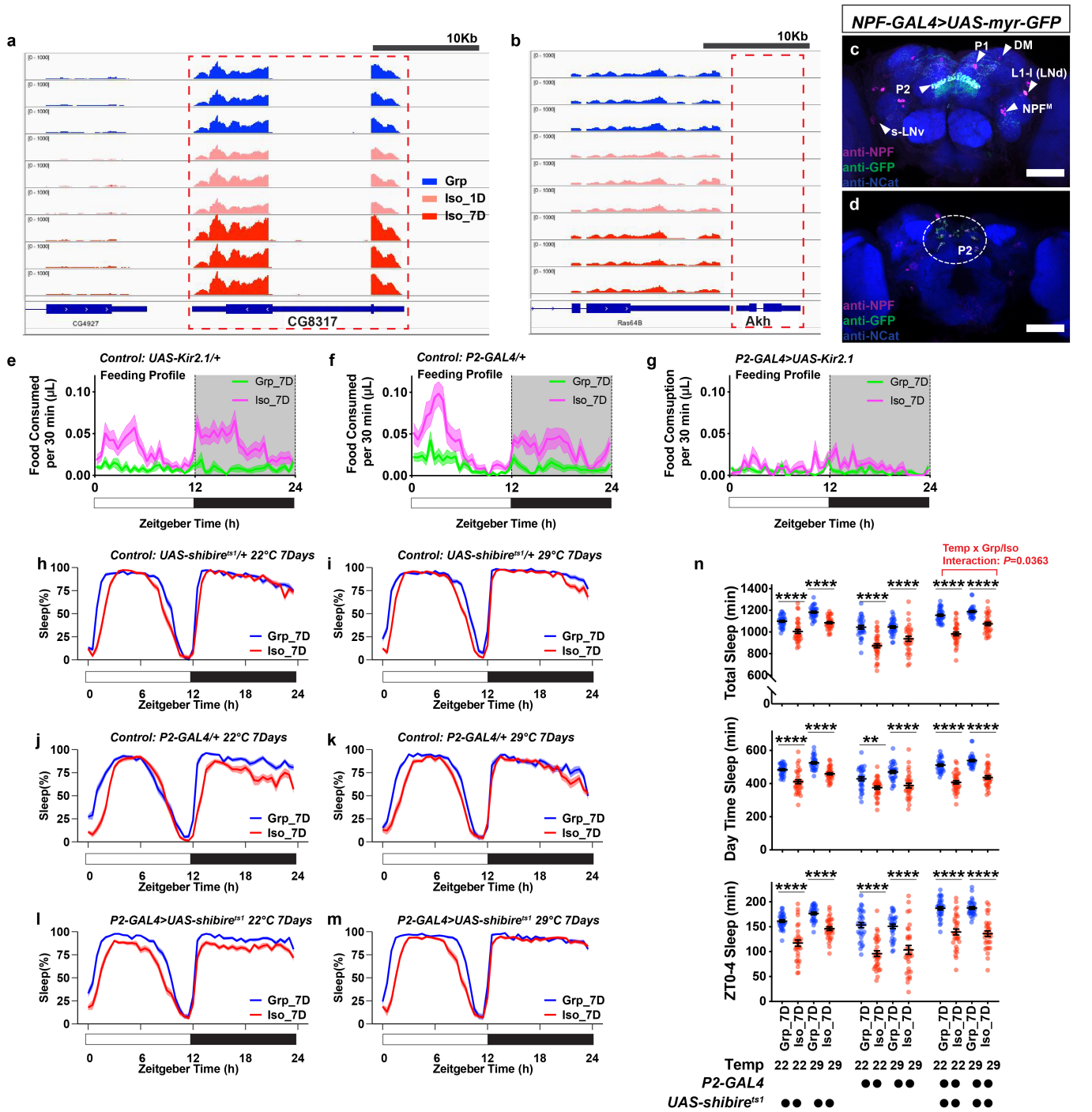
Extended Data Fig. 4 | Social isolation reduces sleep in *Drosophila* SIP lines. **a**–**d**, Sleep profiles of long-sleeping flies: SIP-L1-3 (**a**), SIP-L1-4 (**b**), SIP-L2-1 (**c**), and SIP-L2-6 (**d**) after group enrichment or social isolation (25 flies in a group for group treatment) for 7 days. **e**–**h**, Sleep profiles of short-sleeping flies: SIP-S1-1 (**e**), SIP-S1-9 (**f**), SIP-S2-3 (**g**), and SIP-S2-9 (**h**) after group enrichment or social isolation (25 flies in a group for group treatment) for 7 days. Sleep profiles are displayed as the mean ± s.e.m. proportion of time spent sleeping in consecutive 30-min segments during a 24-h LD cycle. The long-sleeping and short-sleeping fly lines were randomly selected from the SIP, a panel of inbred *Drosophila melanogaster* strains with extreme long or short sleep-duration phenotypes[35,36].



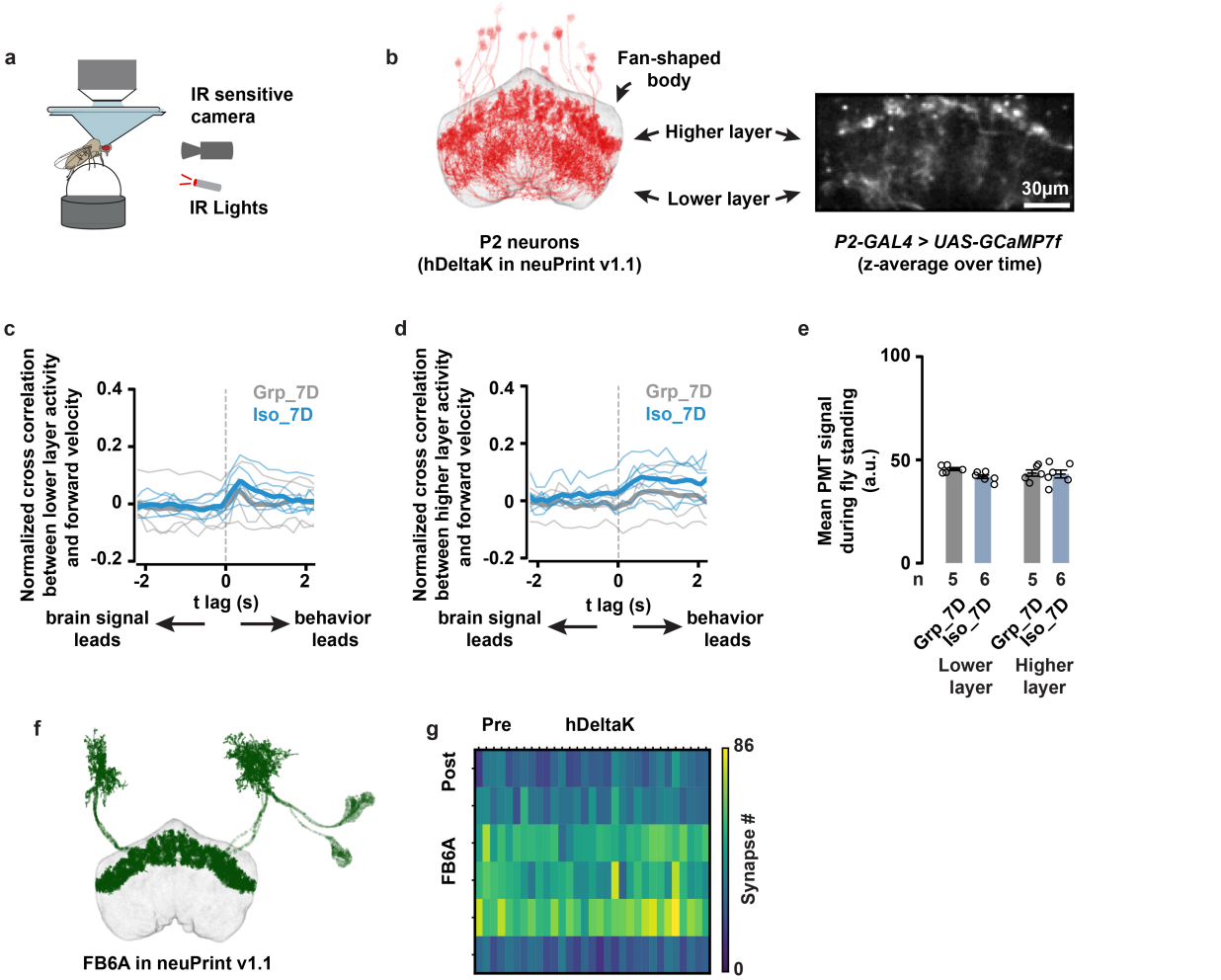
Extended Data Fig. 5 | RNA-seq reveals changes in gene expression during chronic social isolation. a, **b**, Volcano plots of differential gene expression from RNA-seq results. **a**, Comparison between chronic isolation and group conditions (Iso_7D vs Grp). **b**, Comparison between chronic isolation and acute isolation conditions (Iso_7D vs Iso_1D). Red dots indicate genes with significant adjusted *P* values in both comparisons. Differential gene expression analyses were conducted using DESeq2, which uses a two-sided Wald test and Benjamini–Hochberg correction. **c**, Venn diagram showing the intersection of the above two comparisons. **d**, Heatmap of the 274 intersected genes showing significant differential gene expression changes in both comparisons. **e**, Gene ontology of the 214 candidate genes from categories II and IV in **d**. Black bar, counts of genes in each GO term; white bar, $-\log_{10}P$ values for each GO term. See Methods and Supplementary Information for details on RNA-seq data analyses.



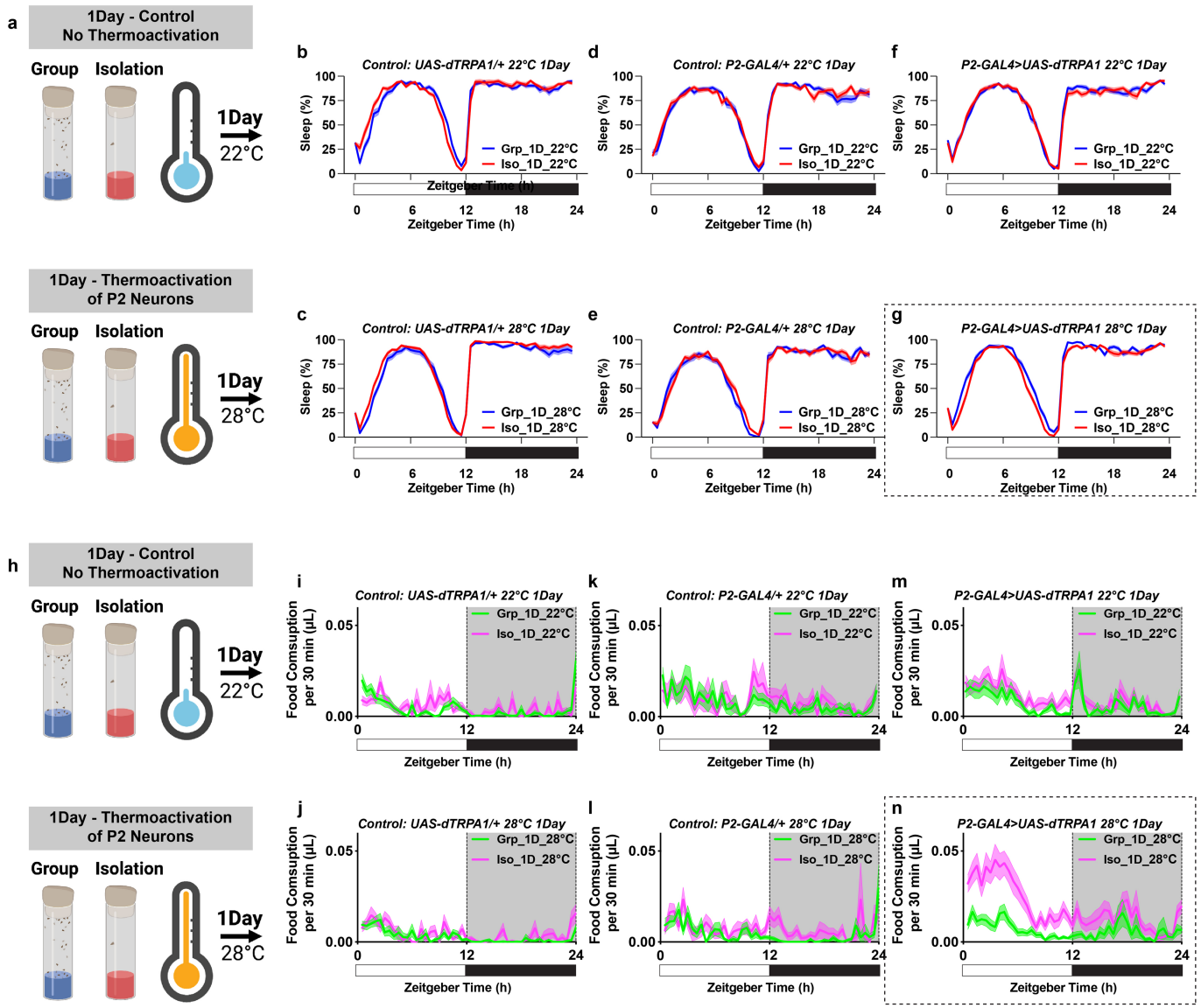
Extended Data Fig. 6 | Chronic social isolation results in reduced sleep and excessive feeding, whereas food consumption is not altered in sleep mutants or after acute social isolation. a, Sleep profiles and matching feeding profiles of three representative individual flies after seven days of group enrichment. **b**, Sleep profiles and matching feeding profiles of three representative individual flies after seven days of social isolation. Sleep profile is presented as sleep amount (min) in consecutive 30-min segments during a 24-h LD cycle. Matching feeding profile is presented as food consumption (µl) in consecutive 30-min segments during a 24-h LD cycle. **c**, Quantification of daily total food consumption, daytime food consumption, nighttime food consumption and ZT0–4 food consumption for wild-type and sleep mutant *inc*^{1} flies (n = 25–29 flies). **d**, Quantification of daily total food consumption, daytime food consumption, nighttime food consumption and ZT0–4 food consumption for wild-type and sleep mutant *fmn* flies (n = 25–29 animals). **e**, Quantification of daily total food consumption, daytime food consumption, nighttime food consumption and ZT0–4 food consumption for wild-type and sleep mutant *wake*^{D2} flies (n = 23–30 flies). **f**, Feeding profile measured by ARC assay in flies following 1 day of group enrichment or social isolation (mean ± s.e.m.). **g**, Quantification of daily total food consumption, daytime food consumption, nighttime food consumption and ZT0–4 food consumption for flies after 1 day of group enrichment or social isolation (**f**, **g**, n = 49–50 flies). All quantifications are displayed as mean ± s.e.m. with individual data points. Unpaired t-tests with Welch's correction. For n and P values, see Source Data.



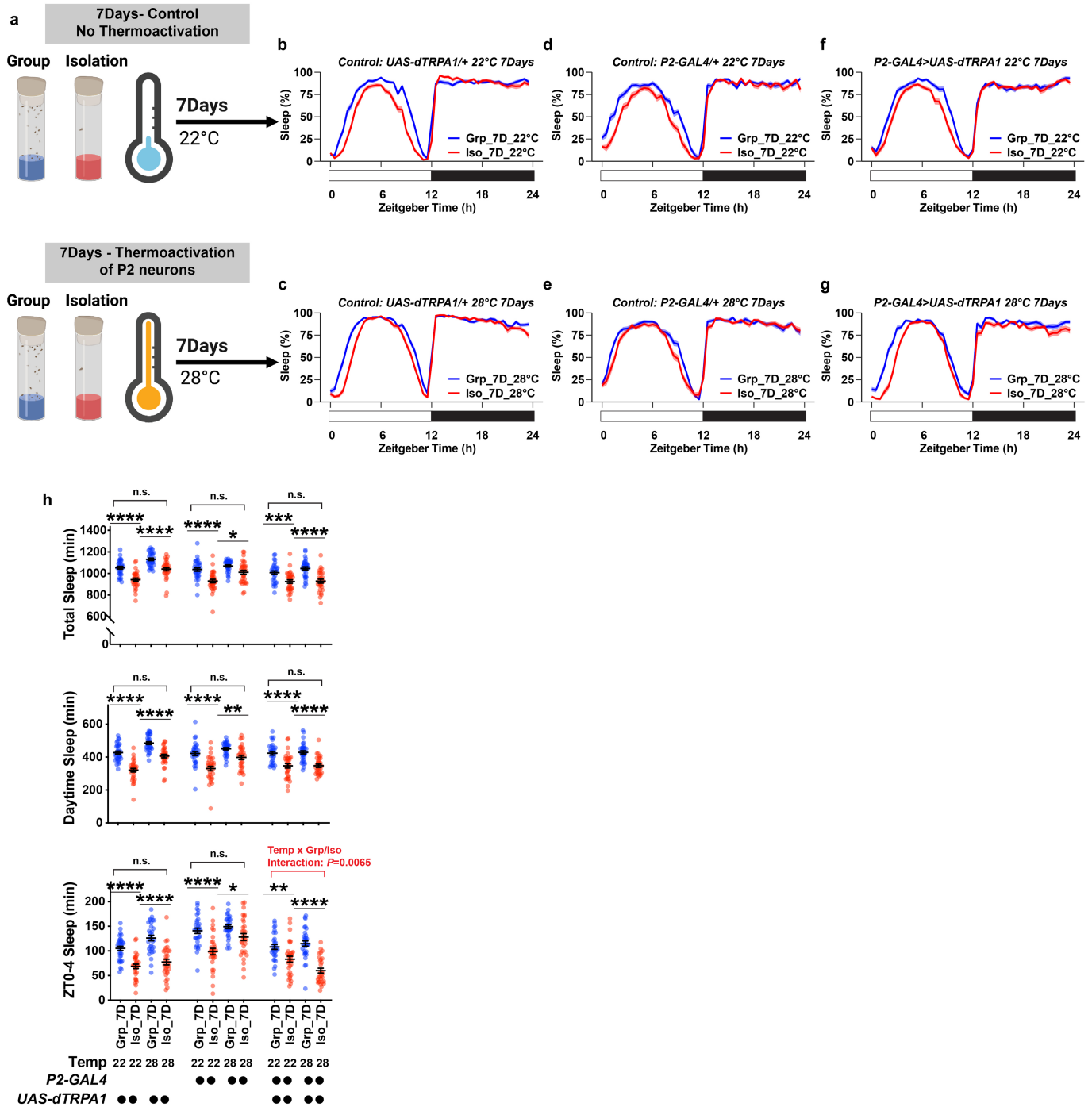
Extended Data Fig. 7 | Limostatin transcripts are detected in fly head RNA-seq sample libraries; *NPF-GAL4* expression pattern; feeding profile of flies in experiments silencing P2 neurons; and silencing P2 neurons with *UAS-shibire^{ts1}* during social isolation is insufficient to block chronic social isolation-induced sleep loss. a, Reads from RNA-seq sample libraries (Grp, Iso_1D, and Iso_7D) align to the gene region of *Lst* (CG8317). **b**, *Akh* and *Lst* are known to be co-expressed in the corpora cardiaca[13]. No reads were detected or aligned to the gene region of *Akh*, suggesting that the RNA-seq samples were free of corpora cardiaca materials and that the measured *Lst* transcripts come from sources in the brain. **c**, Expression pattern of *NPF-GAL4*-labelled neurons revealed by *UAS-myr::GFP* and NPF antibody staining. NPF$^+$ cells overlap with GFP$^+$ cells. P1, P2, DM, L1-l (or LNd)[22], s-LNv[44] and NPF^M (ref. [45]) neurons are labelled. **d**, An additional brain imaged from the posterior end to show NPF$^+$ and GFP$^+$ cell bodies of P2 neurons (dashed circle). Magenta, NPF; green, GFP; blue, N-cadherin; scale bars, 50 µm. **e**–**g**, Feeding profiles measured by ARC assay for parental control flies (**e**, **f**) and flies expressing *UAS-Kir2.1* with *P2-GAL4* (**g**) following 7 days of group enrichment or social isolation (mean ± s.e.m.; $n = 27$–30 flies). **h**–**m**, Sleep profiles for parental control flies (**h**–**k**) and flies expressing *UAS-shibire^{ts}* with *P2-GAL4* (**l**, **m**) following 7 days of group enrichment or social isolation at 22 °C (**h**, **j**, **l**) or 29 °C (**i**, **k**, **m**). All sleep behaviour was tested at 22 °C. **n**, Quantification (mean ± s.e.m. with individual data points) of daily total sleep, daytime sleep and ZT0–4 sleep for parental control flies and flies expressing *UAS-shibire^{ts}* with *P2-GAL4* following 7 days of group enrichment or social isolation at 22 °C or 29 °C. Sleep profiles are displayed as the mean ± s.e.m. proportion of time spent sleeping in consecutive 30-min segments during a 24-h LD cycle. For **h**–**n**, $n = 31$–32 flies; two-way ANOVAs were used for detecting interactions between temperature treatment and group/isolation status; Šidák multiple comparisons tests were used for post hoc analyses between group-treated and isolated animals of the same genotype and temperature treatment; $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$. For *n* and *P* values, see Source Data.



Extended Data Fig. 8 | P2 neurons show similar activity patterns after chronic social isolation or group enrichment and P2 neurons synapse onto cell types labelled by *R23E20-GAL4*. **a**, Tethered, walking, [Ca^{2+}]-imaging setup with an infrared-sensitive camera that tracks the rotation of the ball. **b**, The anatomy of hDeltaK cells from neuPrint[26] (left), compared with time-averaged *z*-projection of GCaMP7f signals driven by *P2-GAL4* (right). Both images show two separate layers (higher layer and lower layer) of fan-shaped body neuropils. **c**, Cross-correlation analysis of the lower-layer GCaMP7f activity and the fly's forward walking velocity. Thin lines, individual fly data; thick lines, population means. **d**, Cross-correlation analysis of the higher-layer GCaMP7f activity and the fly's forward walking velocity. Thin lines, individual fly data; thick lines, population means. **e**, Quantification (mean ± s.e.m. with individual data points) of GCaMP7f activity during standing moments of flies after 7 days of group enrichment or social isolation. Identical two-photon acquisition parameters were used in all experiments (**c**–**e**, $n = 5$–6 flies). **f**, The anatomy of FB6A cells from neuPrint. FB6A has been identified as one of the few cell types labelled by *R23E10-GAL4*[27]. **g**, Synapse-number matrix for detected synapses from P2 neurons (named hDeltaK cells in neuPrint) to FB6A cells. Connectivity data and cell-type names are based on those in neuPrint, hemibrain: v1.1[26].



Extended Data Fig. 9 | Sleep profiles and feeding profiles of flies in which P2 neurons were thermally activated by expressing *UAS-dTPRA1* during acute (1 day) group enrichment or social isolation; parental and temperature controls are included. a, Schematics of activating P2 neurons for 1 day of group enrichment or social isolation. Treatment at 22 °C (no thermoactivation) was used as control. Flies in group enrichment or social isolation were kept at 28 °C for 1 day to thermally activate P2 neurons. After 1 day of thermal activation (or no activation), sleep behaviour was measured at 22 °C. **b**, **c**, Sleep profiles of *UAS-dTRPA1/+* heterozygous control flies after group enrichment or social isolation at 22 °C for 1 day or at 28 °C for 1 day. **d**, **e**, Sleep profiles of *P2-GAL4/+* heterozygous control flies after group enrichment or social isolation at 22 °C for 1 day or at 28 °C for 1 day. **f**, **g**, Sleep profiles of flies expressing *UAS-dTRPA1* under the control of *P2-GAL4* after group enrichment or social isolation at 22 °C for 1 day or at 28 °C for 1 day. **h**, Schematics of activating P2 neurons for 1 day of group enrichment or social isolation. Treatment at 22 °C (no thermoactivation) was used as control. Flies in group enrichment or social isolation were kept at 28 °C for 1 day to thermally activate P2 neurons. After 1 day of thermal activation (or no activation), feeding behaviour was measured at 22 °C. **i**, **j**, Feeding profiles of *UAS-dTRPA1/+* heterozygous control flies after group enrichment or social isolation at 22 °C for 7 days or at 28 °C for 7 days. **k**, **l**, Feeding profiles of *P2-GAL4/+* heterozygous control flies after group enrichment or social isolation at 22 °C for 7 days or at 28 °C for 7 days. **m**, **n**, Feeding profiles of flies expressing *UAS-dTRPA1* under the control of *P2-GAL4* after group enrichment or social isolation at 22 °C for 7 days or at 28 °C for 7 days. Sleep profiles are displayed as the mean ± s.e.m. proportion of time spent sleeping in consecutive 30-min segments during a 24-h LD cycle. Feeding profiles are presented as mean ± s.e.m. food consumption (µl) in consecutive 30-min segments during a 24-h LD cycle. **b**–**e**, **i**–**n**, $n = 28$–32 flies.



Extended Data Fig. 10 | Sleep profiles of flies in which P2 neurons were thermally activated by expressing *UAS-dTPRA1* during chronic (7 days) group enrichment or social isolation; parental and temperature controls are included. a, Schematics of activating P2 neurons for 7 days of group enrichment or social isolation. Treatment at 22 °C (no thermoactivation) was used as control. Flies in group enrichment or social isolation were kept at 28 °C for 7 days to thermally activate P2 neurons. After 7 days of thermal activation (or no activation), sleep behaviour was measured at 22 °C. **b**, **c**, Sleep profiles of *UAS-dTRPA1/+* heterozygous control flies after group enrichment or social isolation at 22 °C for 7 days or at 28 °C for 7 days. **d**, **e**, Sleep profiles of *P2-GAL4/+* heterozygous control flies after group enrichment or social isolation at 22 °C for 7 days or at 28 °C for 7 days. **f**, **g**, Sleep profiles of flies expressing *UAS-dTRPA1* under the control of *P2-GAL4* after group enrichment or social isolation at 22 °C for 7 days or at 28 °C for 7 days. **h**, Quantification (mean ± s.e.m. with individual data points) of daily total sleep, daytime sleep and ZT0–4 sleep for experimental and heterozygous control flies grouped or isolated for 7 days with (28 °C) or without (22 °C) thermal activation of *P2-GAL4*-labelled neurons. Sleep profiles are displayed as the mean ± s.e.m. proportion of time spent sleeping in consecutive 30-min segments during a 24-h LD cycle. For **h**, two-way ANOVAs were used for detecting interactions between temperature treatment and group/isolation status. Šidák multiple comparisons tests were used for post hoc analyses between group-treated and isolated animals of the same genotype and temperature treatment. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, ****$P < 0.0001$; **b**–**h**, $n = 29$–32 animals. For n and P values, see Source Data.

nature research

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Last updated by author(s): Jun 15, 2021

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Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

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☐	☒	The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement
☐	☒	A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
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☒	☐	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.*
☒	☐	For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
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☒	☐	Estimates of effect sizes (e.g. Cohen's *d*, Pearson's *r*), indicating how they were calculated

Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection	Locomotor activity of flies was monitored using the Drosophila Activity Monitor (DAM) system (TriKinetics, Waltham, MA). To measure sleep and feeding behavior simultaneously, ARC (Activity Recording Capillary Feeder) assay (protocol, software and code described in Murphy et al., 2017) was used. Acquisition of frames at 1Hz were performed with Open CV (The MacPorts Project). The object tracking was performed using JavaGrinders (Oracle). Confocal image data were collected on a Zeiss LSM710 confocal microscope. Calcium imaging data were collected on a Bruker two-photon microscope using Prairie View (Bruker). RNA-sequencing data were collected on a Illumina NextSeq 500 sequencer using NextSeq Control Software v1.4.
Data analysis	DAM system data were analyzed with the "R_3.6/Rethomics" package (Geissmann et al., 2019). Density plot for sleep bouts distribution analysis was generated using R/ggplot2_3.3.3 package. ARC system data were analyzed with the Python_3.5/Noah_15.8 script (Murphy, et al., 2017). Commercially available Prism 8 (GraphPad) was used for plotting data and performing statistical analyses. Commercially available Zen 2.3 SP1 software (Zeiss) and publicly available FIJI_2.0.0 software (NIH) were used for processing imaging data. RNA-sequencing reads were aligned to the FlyBase release 6.13 (May 2016) genome assembly with STAR_2.4.2a (Dobin et al., 2013) and read counts were generated with R/featureCounts_1.5.0 (Liao et al., 2014). Differential expression analysis was performed with R/DESeq2_1.26.0 (Love et al., 2014). Gene ontology (GO) analysis was performed using the Database for Annotation, Visualization and Integrated Discovery (DAVID_6.8) (Huang et al., 2009).

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors/reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research guidelines for submitting code & software for further information.

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 RNA-Seq datasets generated in this study have been deposited in NCBI's Gene Expression Omnibus and are accessible through GEO series accession number GSE137498.

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.

Sample size	Sample sizes in behavioral experiments were chosen empirically. Prior research suggested that a sample sizes of n=~32 can detect 2hr differences in daily sleep with a power of 0.9 (Kempf et al., 2019). Comparable sample sizes for each genotype/condition were used in every experiment. For sample size information, see each figure, extended figure legend and source data.
Data exclusions	Dead flies, flies that failed to consume any liquid food from the microcapillaries and flies housed in chambers exhibiting tracking errors were excluded from the analyses.
Replication	All experiments were replicated at least twice independently at different time with different batch of flies to ensure reliable conclusion. All attempts at replication were successful. Replicates were included if necessary, for example to account for variability resulting from incubator temperature fluctuations or food batch variation.
Randomization	Control and experimental flies were reared in identical conditions, and were randomized whenever possible (location in housing incubator, animal selection, position in behavior monitor systems, etc.). Group vs. Isolated animals of the same genotype/condition were always tested in parallel (same batch of fly food, same incubator, same testing period, etc.)
Blinding	Due to the unambiguous nature of measurements in behavioral experiments, investigators were not blinded to fly genotype/condition. Data analyzer was blinded when assessing the results. All data collection and analyses were performed automatically by softwares.

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.

Materials & experimental systems

n/a	Involved in the study
☐	☒ Antibodies
☒	☐ Eukaryotic cell lines
☒	☐ Palaeontology
☐	☒ Animals and other organisms
☒	☐ Human research participants
☒	☐ Clinical data

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☒	☐ Flow cytometry
☒	☐ MRI-based neuroimaging

Antibodies

Antibodies used	Rabbit anti-GFP (Thermo Fisher Scientific, A6455), Mouse anti-GFP (Thermo Fisher Scientific, A11120), Mouse anti-Limostatin (gift from Dr. Seung Kim, Stanford University), Rabbit anti-NPF (RayBiotech, RB-19-0001), Rat anti-N-cadherin (Developmental Studies Hybridoma Bank, DN-Ex #8), Rat anti-FLAG-Tag (Novus, NBP1-06712SS), Rabbit anti-HA-Tag (Cell Signaling Technology, C29F4), Mouse anti-Bruchpilot (Developmental Studies Hybridoma Bank, nc82), Alexa Fluor 488 anti-Rabbit (Jackson ImmunoResearch, 111-545-144), Alexa Fluor 488 anti-Mouse (Thermo Fisher Scientific, A11029), Alexa Fluor 568 anti-Mouse

(Thermo Fisher Scientific, A11031), Alexa Fluor 568 anti-Rat (Thermo Fisher Scientific ,A11077), Alexa Fluor 647 anti-Rat (Jackson Immunoresearch, 112-605-167) and Alexa Fluor Plus 647 anti-Rabbit (Thermo Fisher Scientific, A32733).

Validation

anti-NPF validated in Shao et al., 2017; anti-LST optimized and validated in Alfa et al., 2015; anti-FLAG-Tag and anti-HA-Tag validated in Nern et al., 2015; anti-N-cadherin , anti-Bruchpilot and anti-GFP antibodies are commercially available and widely used and repeatedly validated by the Drosophila scientific research community (previously used in Shao et al., 2017, Aso et al., 2009 and Aso et al., 2014).

Animals and other organisms

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

Laboratory animals	Male Drosophila melanogaster strains of 3-5 days, or 4 weeks after eclosion were used in the study. Detailed information on strain, sex, age at time of testing, transgenes, transgene combinations, and mutations described in Method section.
Wild animals	This study did not involve wild animals.
Field-collected samples	This study did not involve field-collected samples.
Ethics oversight	This study did not require ethical approval.

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