Supplementary Methods

Animal experiments (long)

Animal experiments were conducted in accordance with the German Law for Animal Protection and FELASA’s guidelines for animal research and assessed by the ethical committee of the Ministry of Energy Change, Rural Areas & Consumer Protection (MELUR) of the State of Schleswig-Holstein (licenses 4(76-6/17), 4(99-11/13)). Male mice (C57BL/6J, PER2::LUC/C57BL/6J) were housed under constant darkness conditions (DD; behavioral experiments) or a 12-hour light: 12-hour dark cycle (LD12:12; slice cultures and molecular experiments) in artificially ventilated rooms. Water and high-fat diet (HFD, D12492i, Research Diets, New Brunswick, NJ, US; 60% energy from fat) were accessible ad libitum. Mice were group-housed from 3-4 weeks after birth until one week before the start of experiments. Three days before surgery, mice received liquid diet (LiD, Nutricia Fortimel Compact Vanilla Flavor, Danone, Paris, FR; 2.4 kcal/ml) as a choice. HFD was removed two days prior to surgery and mice were fasted overnight before surgery. After surgery mice received LiD exclusively for two days, a choice of LiD and HFD for the third day and HFD only from thereon (Fig. 1A). Activity was recorded with custom-made infrared detectors positioned above the cage grid. Activity was analyzed in 15-min bins for periodicity (χ² periodogram analysis), onsets, and total activity using ClockLab (ActiMetrics, Wilmette, IL, US). Feeding events were recorded using automated food monitors (BioDAQ, Research Diets). Feeding bouts (≥ 0.01 g) and meals (≥ 0.02 g separated by ≥ 300 s) were exported in 15-min intervals with BioDAQ Data Viewer (Research Diets) and plotted with ActogramJ (Schmid et al. 2011). Applying the activity onsets determined with ClockLab, meal frequency and meal intake of active and resting phase were determined. Meal bout data were smoothened using a 3-hour running average. Daily profiles of meal frequency per hour (= meal activity) were calculated with ActogramJ. Feeding offset (= 0°) was defined as a decline in feeding events to below 50% of the daily average for more than 3 h. Mean meal activity was plotted in 1-hour bins and analyzed with GraphPad Prism (GraphPad, San Diego, CA, US). Mice that did not use the food hopper and sham controls that did not regain weight were excluded from further analysis.
RNA sequencing (long)

RNA-seq libraries and sequencing were performed at the Transcriptome and Genome Analysis Core Unit, University Medical Center Göttingen (Wilms et al. 2019; Pabel et al. 2020). Briefly, using a standard sensitivity RNA Analysis Kit (DNF-471), 200 ng of total RNA was checked for quality and integrity with Fragment Analyzer (Advanced Analytical, Ames, IA, US). All samples selected for sequencing exhibited an RNA integrity number > 8. Libraries were prepared with the TruSeq RNA Library Preparation Kit (version 2, set A, 48 samples, 12 indexes) and the Illumina RS-1222001 protocol (Illumina, San Diego, CA, US). Optimizations step were performed to increase ligation efficiency (> 94 %) and to avoid PCR duplication artifacts and primer dimers. A fluorometry-based system (QuantiFluor dsDNA System, Promega, Madison, WI, US) was used for quantification of cDNA libraries. Average size (~300 bp) of final cDNA libraries was determined with the dsDNA 905 Reagent Kit (Fragment Analyzer, Advanced Analytical). Pooled libraries were sequenced on a HiSeq 2000 (Illumina) generating 50-bp single-end reads (at 25 Mio reads/sample).

Sequence images were transformed with Illumina software (BaseCaller, Illumina, San Diego, CA, US) to BCL files, which were demultiplexed to fastq files with bcl2fastq (version 2.17.1.14, Dodt et al. 2012). FastQC (version 0.11.5; Babraham Bioinformatics, Cambridge, UK) was used for quality control. Sequence alignments to the Mus musculus (mm10) genome were performed using STAR (version 2.5.2a), and read counting using featureCounts (version 1.5.0-p1). Data procession and analysis were done in R/Bioconductor (version 3.6.3; Gentleman et al. 2004). Normalization of raw counts and analysis of differential gene expression analysis was done with DESeq2 (version 1.24.0; Love et al. 2014). Genes with a Benjamini-Hochberg adjusted p-value < 0.05 were considered as differently expressed.

For rhythm analysis (JTK_CYCLE; FDR-corrected p-values < 0.05, Hughes et al. 2010) only transcripts expressed in all replicates of at least one time point and condition were included. A list consisting of the differentially expressed and differentially rhythmic genes (“differently regulated”) was used for gene ontology (GO) enrichment analysis using the BiNGO plug-in for Cytoscape (Shannon et al. 2003;
Maere et al. 2005). Overrepresented biological processes were detected using hypergeometric tests with FDR correction ($p < 0.05$) against the whole mouse annotation library. The network was filtered to only show nodes with a size $> 16$. Top-10 categories of the enrichment analysis and the central GO terms were labeled. Clusters were determined using the Cytoscape search engine. Phase and amplitude were calculated using JTK_CYCLE. For baseline comparisons, individual gene expression counts were normalized against sham control average, and, for rhythmicity comparisons, against the corresponding group gene average. Diurnal fold change (FC) was defined as difference between maximum and minimum normalized by baseline. Gene lists for pathway analyses were taken from wikipathways.org (WP33, WP336, WP386, WP3588; Slenter et al. 2018). Data sets are accessible through NCBI’s Gene Expression Omnibus (accession number GSE162671).