Extended Materials and Methods

Mice Mice were between 2 and 4 months old at time of behavioral experiments (weighing between 25-30g). Mice were typically group housed for acquisition, retrieval, and consolidation experiments, although a cohort of single-housed mice were used in one replication of the first experiment (Fig. 1). As all ZT groups were equally represented in this replication and no behavioral differences were observed between housing conditions in any phase of the experiment (e.g. Testing: Two-way ANOVA, significant effect of ZT Time ($F_{(5,57)}$=3.558, p<0.01), no effect of Housing or Interaction), data for single- and group-housed mice were collapsed (Fig. S5). For the circadian rhythm/sleep experiment (Fig. 5), mice were single-housed to enable accurate measurement of each individual animal's movement.

Object Location Memory (OLM) Mice were handled for 2 min/day for 4 days and habituated to the context for 5 min/day for 6 days. For training, mice were exposed to two identical objects (100 mL beakers filled with cement) for 10 minutes. For testing, mice were returned to the context for 5 minutes with one object moved to a new location. For long-term memory tests, mice were placed back into the context 24 or 36 hours later and for short-term memory tests, mice were placed back into the context 60 minutes later. Object exploration was quantified by blinded experimenters and exploration was defined as the mouse orienting to the center of the object and within 1 cm of the object itself. Total time spent investigating was quantified with DeepEthogram [1], with the scoring parameters chosen to closely match handscoring methods previously used to score OLM behavior [2–4]. A discrimination index (DI) was calculated based off the amount of time spent investigating the novel location in comparison to the original location. Mice that explored <2.5s during testing, <4s during training, or that showed an existing object preference (DI > ±20) during training were removed from all analyses. Habituation sessions were analyzed for distance traveled and speed using Ethovision (Noldus). For molecular experiments, mice were sacrificed via cervical dislocation 60 minutes following training and the brain was harvested and flash-frozen in isopentane. For all OLM experiments, habituation, training, and testing were conducted under dim red light.

RT-qPCR Tissue was harvested from DH punches and frozen at -80°C until use. RNA was extracted using the RNeasy Minikit (Qiagen). cDNA was synthesized using the Hi-Fi cDNA Synthesis Kit (Abcam). To reduce the potential for genomic DNA amplification, assays were designed to be intron spanning for the *Per1* target assay and the *GAPDH* reference assay (IDT) designed based on the Mus_musculus, GRCm38 build. qPCR was run on the Roche LightCycler 96 with the following cycling conditions: 1 cycle at 95°C for 3 min, 45 cycles of 95°C for 15 sec and 60°C for 60 sec, and hold at 4°C. Data were analyzed with LightCycler Analysis software using the Relative Quantification analysis method. For *Per1* we used the following primers: left 5'-CCTGGAGGAATTGGAGCATATC-3'; right 5'-CCTGCCTGCTCCGAAATATAG-3'; probe 5'-AAACCAGGACACCTTCTCTGTGGC-3' labeled with FAM. We used *Gapdh* as

a reference assay with the following primers: left 5'-GGAGAAACCTGCCAAGTATGA-3'; right 5'-TCCTCAGTGTAGCCCAAGA-3'; probe 5'-TCAAGAAGGTGGTGAAGCAGGCAT-3' labeled with HEX.

RNA-Sequencing RNA was extracted from DH 500 µm punches and total RNA quality and concentration was evaluated with an RNA High Sensitivity Screen Tape on a TapeStation 4150 (Agilent). A uniquely indexed cDNA library was prepared by the Penn State Genomics Core according to the TruSeq Stranded mRNA kit according to manufacturer's protocol (Illumina) using 200 ng for each sample. Library quality was assessed with the DNA 5000 Screen Tape for TapeStation 4150 and concentration was determined with qPCR using the KAPA Library Quantification Kit for Illumina platforms (Kapa Biosystems). A 10 nM equimolar pool of all libraries was made and confirmed by sequencing with MiSeq Nano 150 x 150 paired-end sequencing. Each pool was sequenced on an Illumina NovaSeq instrument during a single-read 100 bp sequencing, run by the Genome Sciences Facility at the Penn State College of Medicine. The resulting sequencing data for each library were post-processed to produce FastQ files. Data consisted of 8-53 bp paired end reads. FastQC reports were generated to check the data quality. Data were mapped to the reference genome (Mus_musculus, GRCm38 build) using hisat2 (v2.1.0). The average mapping rate was 95%. Mapped data was visualized and inspected in IGV (Integrative Genomics Viewer). Reads that mapped to the genes were counted using featureCounts (v2.0.0) specifying -s 2 -p parameters. Transcript level counts were obtained using Kallisto (v0.46.1). Raw reads were normalized and differentially expressed genes and transcripts were obtained using DESeq2 package. Circadian genes were identified with JTK algorithm [5]. JTK_cycle [5] is a program that can detect rhythmic patterns from time-series data. It is based on Jonckheere-Terpstra-Kendall (JTK) test, which is a non-parametric statistical test to detect trends or cyclic patterns in data over time. The test statistic measures the degree of order or trend in the data and a p-value is calculated to determine the significance of the trend. We utilized the JTK algorithm implemented in R's MetaCycle [6] library. Pathway analyses were preformed using the KEGG database. Over-representation analysis with clusterProfiler package in R was used to identify enriched KEGG pathways.

HSV Production Neuron-specific herpes simplex viruses (HSVs) were used to express the CRISPR interference (CRISPRi) system (dCas9-KRAB-MeCP2) and the single guide RNA (sgRNA), either targeting *Per1* or a non-targeting control. All viruses were purchased from Dr. Rachael Neve (Gene Delivery Technology Core, Massachusetts General Hospital, Boston, MA). CRISPRi was expressed in a p1005 vector that simultaneously expresses a deactivated Cas9 (dCas9)-KRAB-MeCP2 fusion under the hSyn promoter along with mCherry (via an IRES element). The *Per1* sgRNA was previously designed and chosen based on its efficacy in reducing *Per1* in HT22 cell culture and has previously been shown to locally reduce *Per1* in the brain *in vivo* [7]. The *Per1* sgRNA or the non-targeting control sgRNA was cloned in the pDonr221 vector (ThermoFisher) containing the U6 promoter and the sgRNA sequence. Dr. Neve

subcloned the inserts into an HSV vector with GFP under the CMV promoter. The sgRNA sequences were as follows: *Per1*: GAGTTCGACGGCTCCAGAGTA and non-targeting: GCGAGGTATTCGGCTCCGCG. The non-targeting sgRNA was previously validated in similar CRISPR systems [8].

Stereotaxic Surgery Mice were anesthetized with 1.5%-4% isoflurane and placed in the stereotaxic apparatus. After leveling the skull, Bregma was measured and holes were drilled through the skull above the dorsal hippocampus (DH; AP, −2.0 mm; ML, ± 1.5 mm, DV, −1.5 mm relative to Bregma). Bilateral infusion needles were lowered to the DH at a rate of 0.2 mm/15 s. Two minutes after reaching the DH, 2.0 µL of an equal parts mixture of HSV-CRISPRi and HSV-sgRNA-*Per1* or HSV-sgRNA-ctrl was infused into each hemisphere at a rate of 10 µL/h. Post-infusion, infusion needles remained in place for 5 minutes, then moved up 0.1 mm and remained in place for an additional 5 minutes prior to being removed at a rate of 0.1 mm/15 s.

Circadian Rhythm and Sleep Time Analysis Mice were single housed under a 12 h light/dark (LD) cycle and their activity was continuously monitored with passive infrared sensors and Clocklab software (both Actimetrics). After a 2-week entrainment period, mice were infused with HSV-CRISPRi and either the HSV-sgRNA-*Per1* or HSV-sgRNA-ctrl (described above) to reduce *Per1* in the dorsal hippocampus. Mice were given 2 days of recovery to enable the virus to fully express before being placed in constant darkness (DD) for 10 days (after which the HSV is fully eliminated [9,10]). Tau values were calculated using the onset of free activity and calculating the least-squares fit with Clocklab software. Data obtained between surgery and constant darkness was not included in the analysis, as mice were removed from their homecages and weighed daily during this recovery period, rendering the movement/sleep data inaccurate. Sleep behavior was assessed using a program adapted from the COMPASS system [11]. Movement data were collected in 10s bins with the criteria that periods of inactivity lasting 40s or longer were quantified as a behavioral correlate of sleep. Previous work has demonstrated a high degree of correlation between this immobility-defined sleep and sleep defined using EEG records [11]. This sleep behavior (in seconds) was combined within 30 minute bins across the day/night cycle.

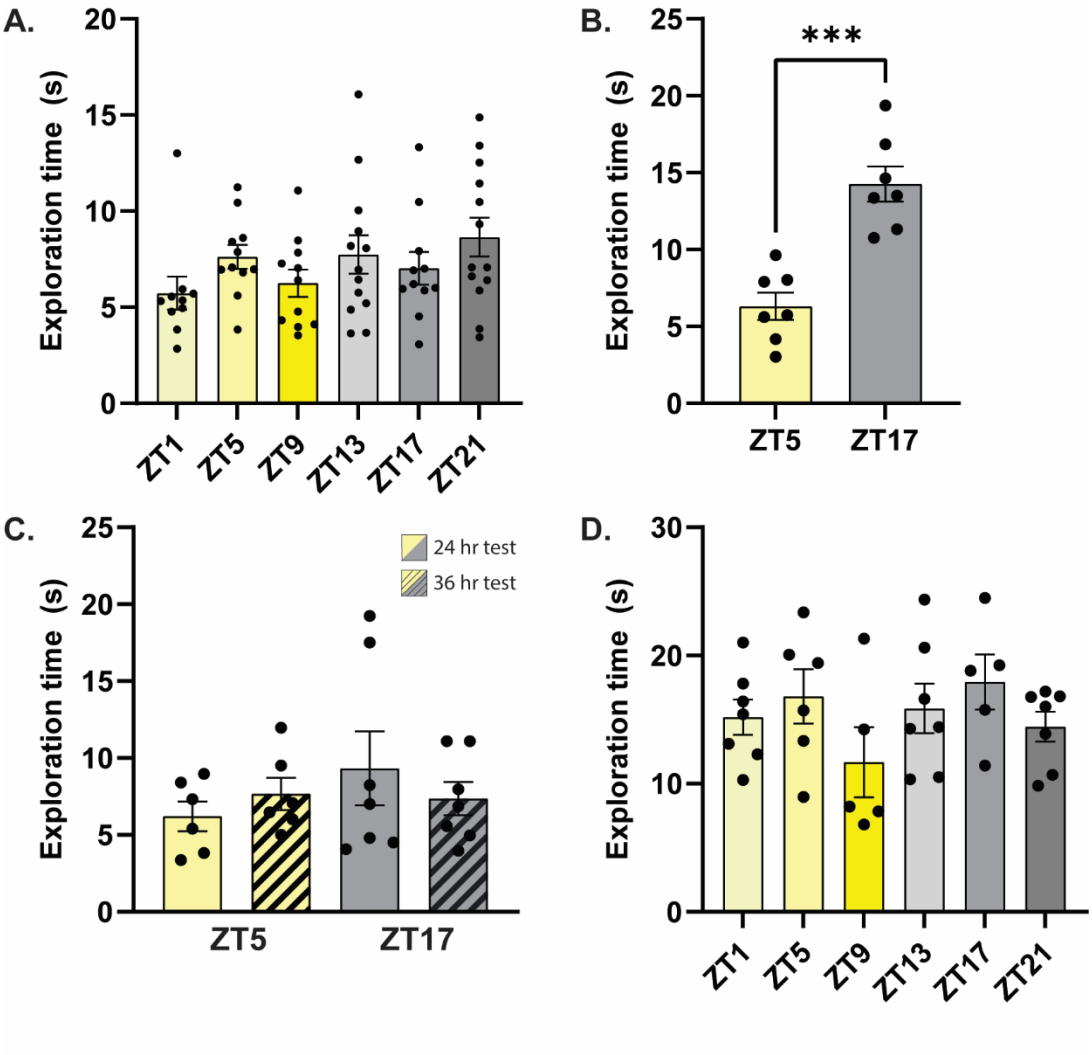

Fig S1. Exploration time does not have an effect on memory performance. A) Total object exploration during the test session for circadian long-term memory experiment (Fig. 1; n=11-13/timepoint). B) Total object exploration during the test session for the short-term memory experiment (Fig. 2; n=7/timepoint). C) Total object exploration during the test session for the retrieval experiment (Fig. 2; n=6-7/timepoint). D) Total object exploration during the training session for the RNA-sequencing experiment (Fig. 3; n=5-7/timepoint). *** = $p<0.001$. ZT = Zeitgeber Time, where ZT0 = 6am (7am DST), lights on, ZT12 = 6pm (7pm DST), lights off.

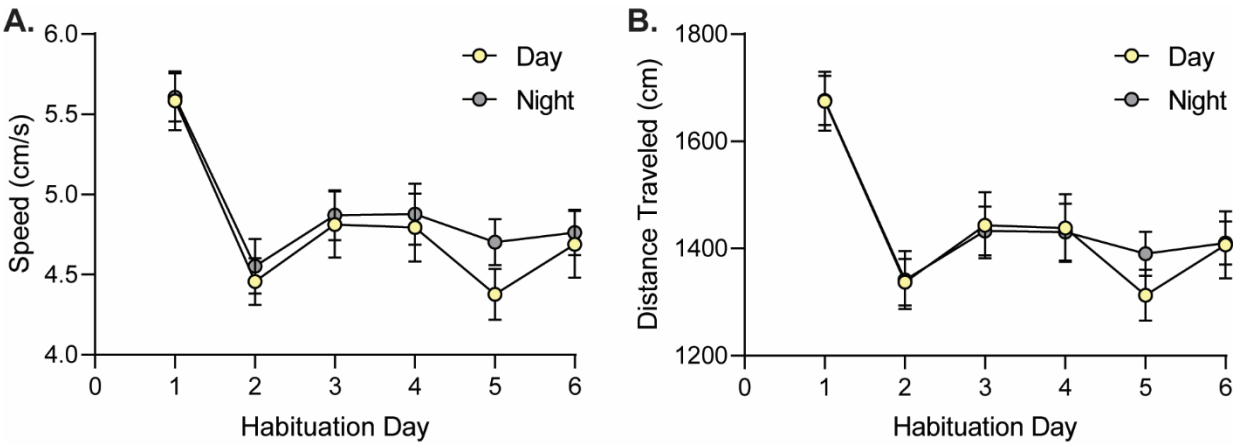

Fig S2. Mouse speed is similar during the day and night. A) Speed, in cm/s, and B) distance traveled, in cm, of mice habituated during the day (yellow symbols; ZT1, ZT5, ZT9) and mice habituated at night (grey symbols; ZT13, ZT17, ZT21) across the 6 days of habituation from the circadian long-term memory experiment (Fig 1; n=18-21/timepoint).

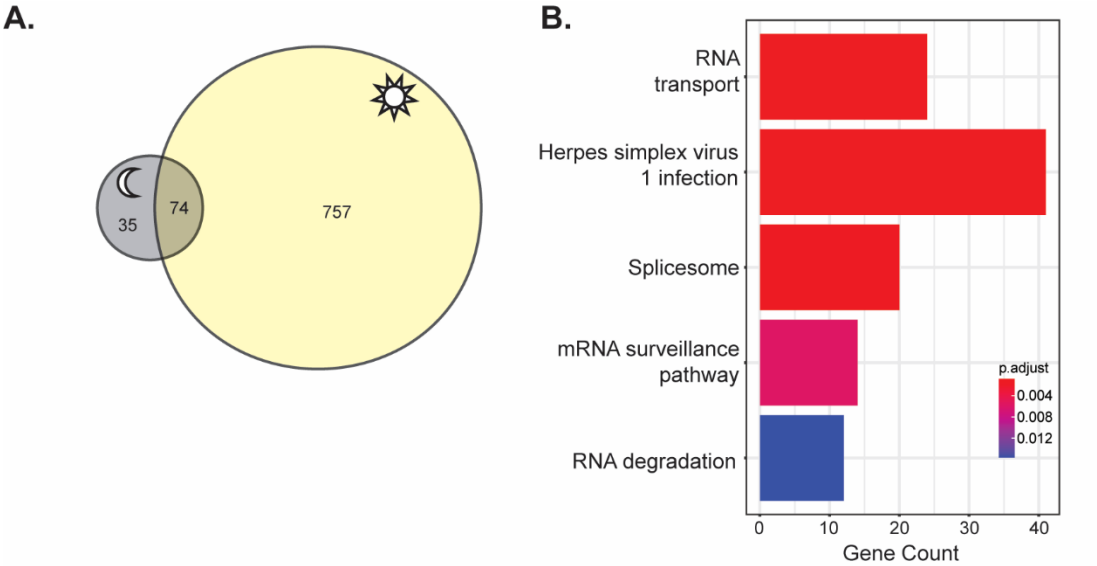

Fig S3. Learning during the day drives major changes in gene expression that do not occur at night. A) Genes upregulated in response to learning during the day (sun symbol), night (moon symbol), or both. B) Top Kegg pathways upregulated during the day but not at night.

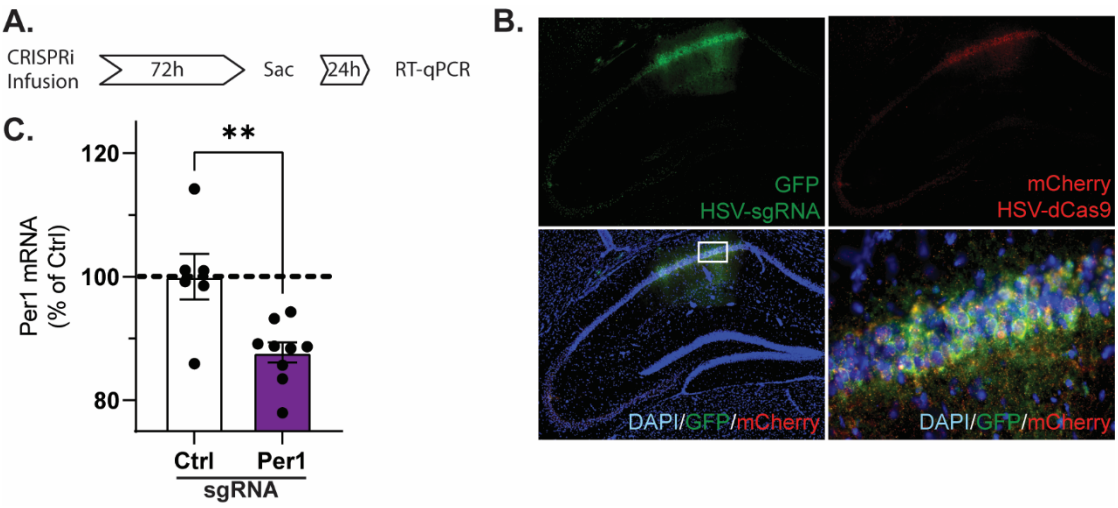

Fig S4. Knock down of Per1 in the dorsal hippocampus. A) Timeline. B) Representative HSV-CRISPRi expression in the DH 3d post-infusion, showing a high degree of co-expression of the sgRNA vector (GFP, top left) and dCas9 (mCherry, top right). Bottom: merged images. C) RT-qPCR shows that HSV-CRISPRi significantly reduces *Per1* expression in vivo (n=6-9/condition). ** = p<0.01.

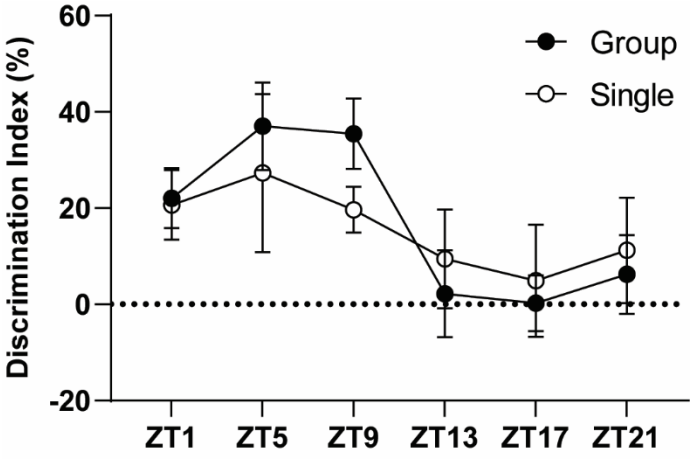

Fig S5. Housing does not have an effect on memory performance. Single-housed mice (white symbols) showed the same memory oscillations over the day/night cycle as the group-housed mice (black symbols). There was no significant difference in memory performance at any timepoint (n=4-8/timepoint). ZT = Zeitgeber Time, where ZT0 = 6am (7am DST), lights on, ZT12 = 6pm (7pm DST), lights off.

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