

Supplementary Materials:

Materials and Methods

Animals, Housing, and Lighting Conditions. Siberian hamsters (*Phodopus sungorus*) were bred in our laboratory in a 16:8-h light-dark (LD) cycle at an ambient temperature of 22°C as described (20). Animals were provided with cotton batting for nesting material; food (Purina chow # 5015) and tap water were available *ad libitum*. All experimental procedures were approved by Stanford University's Administrative Panel on Laboratory Animal Care (Animal Use Protocol Number: 14988) and were conducted in accordance with the NIH Guide for the Care and Use of Laboratory Animals. Light fixtures contained two cool white fluorescent tubes (4,100 K, Philips 40 W) producing an intensity of 10–60 $\mu\text{W}/\text{cm}^2$ on cage floors when water bottles, food, and cage lids were in place.

Activity Recording and Analysis. Activity bouts were summed in 10-min intervals by passive infrared motion detectors. Activity data of animals that appeared arrhythmic based on visual inspection of their actograms were evaluated for circadian periodicity by chi-square periodogram analysis (ClockLab, Actimetrics, Evanston, IL) on 10-day blocks of data for each animal immediately prior to behavioral testing. Peaks in the periodogram were deemed statistically significant if they exceeded the 99.9 % confidence interval limit. Animals were considered arrhythmic if there were no significant peaks in the periodogram in the circadian range, activity was distributed throughout the LD cycle, and if daily rhythm onsets and offsets could not be identified visually. The time of day when animals were tested is given by zeitgeber time (ZT) where ZT 0 = time of lights-on and ZT 16 = time of lights-off in the animal rooms.

Induction of Circadian Arrhythmia. Equal numbers of males and females were used in all groups and were 2-4 months of age at the start of the experiment (20). Hamsters were separated and housed singly in the same photoperiod as the colony room (LD 16:8, lights on at 0200 PST). Rhythms were eliminated using a disruptive phase-shift (DPS) protocol as follows. Fourteen days after being housed singly, lights in the activity recording chambers were turned on for 2 h beginning 5 h after lights-off (i.e., a 2-h light pulse). On the next day, the LD cycle was phase delayed by 3 h so that dark onset occurred 3 h later than on the previous night (lights on at 0500 PST). Animals remained in the 16:8 LD cycle (lights on at 0500 PST) thereafter, and locomotor activity was continuously recorded. Animals with confirmed loss of circadian locomotor rhythms (i.e., arrhythmic, ARR) at 4 weeks after the DPS treatment were randomly assigned to their experimental groups, along with age- and gender-matched controls from the hamster colony (i.e., entrained, ENT).

Surgical Procedures. Animals were anesthetized with pentobarbital sodium (80 mg/kg). The skull was secured and held level in a stereotaxic apparatus. Bilateral radiofrequency lesions were made by heating the tip of a flexible stainless steel electrode (Neurotherm, Wilmington, MA) that was insulated except for 2 mm at its tip to 55 °C for 10 sec. Lesion coordinates were 1.0 mm anterior to bregma, ± 0.2 mm lateral to the sagittal sinus, and 6.6 mm ventral to dura. Sham-operated animals underwent the same procedure except no current was passed through the electrode.

Lesion Verification. Brains were removed and frozen coronal sections (30 μm) cut through the area of the optic chiasm. Mounted sections were stained with cresyl violet, and the extent of the damage was assessed microscopically. Histological evaluation of tissue damage was performed by an independent investigator without knowledge of the corresponding

behavioral data.

Data Analysis. Performance on the NOR and SA tasks was determined by one-sample t-tests to determine whether scores were statistically different from random chance performance (i.e., DI = 0 for NOR; alternations (%) = 50 for SA). A score of positional bias was created to check for differences in left-right biases in the NOR arena or in the T-maze arms. Positional bias was calculated as: time on the right/(time on the left + time on the right) x 100, so that a score that is significantly < 50% indicates a left bias, and > 50% indicates a right bias. Changes in the number of arm entries and exploration time were evaluated by one- or two-way ANOVA (group x time of day) depending on the number of conditions being tested. Data are presented as mean \pm (SEM).

Results

Exploration Behavior:

Experiment 1. The number of arm entries in the T-maze, total exploration time (sec) in the NOR arena, and positional biases in the maze and arena in figure 1 were compared among ENT, DPS, SCNx, and SHAM groups by one-way ANOVA. Data were analyzed with Prism 6.0 (GraphPad Software, Inc.). No differences were found, suggesting that motivation to explore the objects and environments associated with the behavioral tests was constant among the groups. T-maze arm entries: $F_{(3,36)} = 0.63$, $P = 0.610$; exploration time NOR arena: $F_{(3,36)} = 0.39$, $P = 0.761$; positional bias T-maze: $F_{(3,36)} = 2.33$, $P = 0.093$; positional bias NOR arena: $F_{(3,36)} = 1.20$, $P = 0.320$.

Exploration times in the NOR arena are presented by experimental phase (Sample, Test; Fig. S1A). Time spent with the left (L) or right (R) object during the sample phase, or time spent with the novel (N) or familiar (F) object for the test phase, are shown (Fig. S1A). Differences in object exploration times were statistically evaluated by paired t-tests. For all four groups, exploration was divided equally between left and right objects during the sample phase ($P > 0.05$). Significantly more time was spent with the novel object compared to the familiar one during the test phase for ENT (n=10; $P=0.002$), SCNx (n=11; $P=0.009$), and Sham (n=11; $P=0.004$) groups, but not for the DPS animals (n=8; $P > 0.05$; Fig. S1A).

The number of arm choices that constituted an alternation (“A”) or a nonalternation (“N”) in the T-maze are presented for the four experimental groups (Fig. S2A), and statistically compared using paired t-tests. Significant differences were found for ENT (n=10; $P < 0.001$), SCNx (n=11; $P=0.008$), and Sham (n=11; $P=0.005$) groups, but not for the DPS group (n=8; $P > 0.05$), which failed to score more alternations than expected by chance.

Experiment 2. The number of arm entries in the T-maze, total exploration time (sec) in the NOR arena, and positional biases in the maze and arena were compared using two-way ANOVA for surgical condition (SC; SCNx, PSCNx, and SHAM) with repeated measures for rhythm status (RS; ENT, DPS, Lesion). No differences were found, suggesting that motivation to explore the objects and environments associated with the behavioral tests was constant and remained unaffected by surgery or circadian status. T-maze arm entries: [SC: $F_{(2,25)} = 2.73$, $P = 0.092$], [RS: $F_{(2,50)} = 0.04$, $P = 0.962$]; NOR exploration time: [SC: $F_{(2,25)} = 0.76$, $P = 0.476$], [RS: $F_{(2,50)} = 2.74$, $P = 0.074$]; positional bias T-maze: [SC: $F_{(2,25)} = 0.47$, $P = 0.632$], [RS: $F_{(2,50)} = 0.19$, $P = 0.824$]; positional bias NOR arena: [SC: $F_{(2,25)} = 0.007$, $P = 0.993$], [RS: $F_{(2,50)} = 1.05$, $P = 0.360$].

Exploration times in the NOR arena were evaluated by paired t-tests and presented as

described for experiment 1 (Fig. S1B). There were no differences found in exploration times between left and right objects during the NOR sample phase for any of the three groups during ENT, DPS, or Lesion phases of the experiment ($P > 0.05$; Fig. S1B). During the NOR test phase, significantly more time was spent with the novel object versus the familiar one when the animals were rhythmic and entrained (ENT) to the light-dark cycle (Sham, $P=0.002$; PSCNx, $P=0.012$; SCNx, $P=0.020$). Once DPS-arrhythmia was induced, there was no longer an exploration preference for the novel object ($P > 0.05$). After surgery, novel object preference was reinstated only in animals with complete SCN lesions (SCNx; $P=0.006$; Fig. S1B).

The number of arm choices were classified and presented as described for experiment 1 (Fig. S2B). When the animals were entrained (ENT), all groups made significantly more alternations than nonalternations [SHAM, $P < 0.001$; PSCNx, $P=0.007$; SCNx, $P=0.011$]. After the DPS treatment, none of the hamsters exhibited significant alternation behavior ($P > 0.05$). After surgery, only the SCNx animals made significantly more alternations than nonalternations in the T-maze ($P=0.004$; Fig. S2B).

Sleep in DPS Arrhythmic Hamsters:

Sleep plays an important role in memory processing, and so it is worth asking whether our results could be explained by changes in sleep amounts, duration, or fragmentation. In a prior study, we used electroencephalogram (EEG) recordings to evaluate sleep patterns and homeostatic responses to sleep deprivation in arrhythmic DPS-treated animals (28). Several findings from that study are relevant here. DPS animals did not exhibit any masking of sleep by the light-dark cycle as determined by comparing EEG recordings in the light-dark cycle and in constant darkness. Bouts of non-REM (NREM) sleep in arrhythmic hamsters during the light and dark phases of the light-dark cycle did not differ either, and were as long as those expressed by entrained animals during the light phase. Therefore arrhythmic animals slept more during 24 h, by approximately 90 min.

That study also revealed that sleep homeostasis is intact in DPS arrhythmic hamsters (28). Their responses to sleep deprivation periods of 2- or 6-h were no different than the responses of rhythmic entrained animals. The amounts of slow wave activity (SWA) in the EEG during recovery sleep were proportional to the duration of prior waking in both DPS arrhythmic and entrained animals, and were of the same magnitude. These results suggest that sleep homeostasis is not affected by DPS arrhythmia, just as SCN lesions do not impair homeostatic responses to sleep deprivation (29, 30).

Entrained hamsters exhibit a sharp increase in SWA at the onset of the light phase of the light-dark cycle, which declines to basal levels within 4-5 h (2). Once basal sleep levels are reached, the relative amounts of SWA, NREM, and REM sleep, as well as the duration of NREM sleep bouts, are the same as the amounts observed in arrhythmic animals during the remainder of the light phase. These previous findings suggest that sleep pressure is not different between entrained and arrhythmic hamsters at and around the time the NOR and SA tests were performed in the present study. Furthermore, among entrained hamsters, the relative amounts of SWA, NREM, and REM sleep is the same at 7 and 11 h after light onset (i.e., zeitgeber time (ZT) 7 and 11), even though they fail in both the NOR and SA tests at ZT 7, but are successful at ZT 11 (19, 20). Thus, arrhythmic animals do not fail at memory tests because they are either too sleepy or sleep-deprived. Rather, the data suggest that memory processing is under circadian control in a manner that can be separated from sleep effects on memory.

Relationships Between Bouts of Activity and Rest as Approximate Measures of Sleep:

For the present study, we conducted a detailed examination of activity and rest patterns in the home cages of animals from experiment 2 during a 3-h window before and after the NOR sample and test phases to see if it could explain the failure of DPS animals in the memory tests, or the recovery of memory in SCNx animals. Although a lack of activity does not indicate sleep, differences in activity patterns might suggest sleep-related mechanisms that could potentially explain our findings. Locomotor activity across ENT, DPS, and SCNx conditions were analyzed by 2-way ANOVA with repeated measures for treatment condition (Fig. S3). During the 3-h window before the NOR task, there was a significant increase in activity in the time leading up to both the sample ($F_{(17, 102)} = 6.44$, $P < 0.0001$) and test ($F_{(17, 102)} = 5.50$, $P < 0.0001$) phases for all groups. This increased activity is common as it most likely reflects the animal's arousal to being removed and returned to their soundproof chambers. Notice that this bump in activity is lower on the following day (test day) as animals begin to habituate to being transferred from their home to the behavioral test.

Locomotor activity did not change significantly over time in the 3-h period after object exploration for any of the groups during the sample phase ($F_{(17, 102)} = 0.83$, $P = 0.652$) or test phase ($F_{(17, 102)} = 1.71$, $P = 0.052$; Fig. S3). Although activity did not change significantly over time in the test phase, an F value with a marginal significance level (0.052) must be interpreted with caution, as there seems to be an initial suppression of activity lasting ~90 min among all 3 groups. The amounts of locomotor activity before and after both phases of the NOR task did not differ among the ENT, DPS, and SCNx conditions during the sample phase [pre-exploration: ($F_{(2, 12)} = 1.41$, $P = 0.282$); post-exploration: ($F_{(2, 12)} = 0.095$, $P = 0.910$)] or test phase [pre-exploration: ($F_{(2, 12)} = 2.99$, $P = 0.088$); post-exploration: ($F_{(2, 12)} = 0.42$, $P = 0.662$)]. These data suggest that all of the animals, regardless of surgical condition, were awake and vigilant going into each phase of the NOR test and were quiescent afterwards, thereby giving them an opportunity to sleep after training.

Sleep fragmentation can impair memory, but DPS-arrhythmic animals have bouts of NREM sleep that are the same as entrained hamsters in terms of bout duration and amount of sleep at the times of day that we performed the memory tests (28). Thus, sleep fragmentation is unlikely to explain the poor memory performance of the DPS animals, but it might explain the improved performance of SCNx hamsters if they were found to have significantly longer rest periods than the DPS ones. To examine this issue, we analyzed the duration of activity bouts during the 3-h before and after object exploration in the NOR task to see if SCN lesions decreased activity bout duration, which would suggest an increase in sleep bout duration, an effect that could improve memory. Locomotor activity is collected in 10-min bins, so we defined a bout of activity as the number of consecutive 10-min bins with any activity in it. Frequency histograms of the data show that activity was dominated by 10- and 20-min bouts (Fig. S4). We compared the proportions of activity bouts ranging from 10-60 min across the ENT, DPS, and SCNx conditions by using a chi-square analysis. There were no significant differences in the distribution of activity bout duration among the three groups during the sample [pre-exploration: $\chi^2 = 7.18$, $P = 0.709$; post-exploration: $\chi^2 = 4.30$, $P = 0.636$] or test [pre-exploration: $\chi^2 = 12.96$, $P = 0.226$; post-exploration: $\chi^2 = 5.19$, $P = 0.520$] phases of the NOR task (Fig. S4). These data suggest that group activity was consolidated to the same extent around the time the behavioral tests were conducted in the present study, and minimize the possibility that

differences in sleep fragmentation conferred any benefit to animals with SCN lesions.

In general, there is an inverse relationship between rest and locomotor activity over time scales of many hours. We were interested in whether this relationship might hold over much shorter periods because it could potentially explain differences in DPS and SCN_x task performance if DPS animals did not get sufficient rest between bouts of activity, or if SCN_x animals had extended rest periods. Bouts of activity and rest (defined as zero activity) were quantified during the 3-h periods before and after object exploration in the NOR task for the animals from experiment 2. Data were analyzed separately for ENT, DPS, and SCN_x conditions, but pooled across the sample and test phases of the NOR task. Locomotor activity bouts were quantified and correlated with the bout of rest that followed each bout of activity. No significant differences were found in any condition, so the data were pooled from the sample and test phases. Pearson correlations did not reveal significant relationships for any of the conditions (ENT: $r = -0.215$, $P=0.229$, $n=33$; DPS: $r = 0.036$, $P=0.858$, $n=27$; SCN_x: $r = -0.061$, $P=0.728$, $n=35$).

Histological Analysis of Lesioned Animals:

SCN lesions were defined as having no visible SCN tissue remaining. Damage to areas surrounding the SCN included the paraventricular nuclei (PVN), medial preoptic area (MPOA), and the retrochiasmatic area (RCA). Damage to these areas in both SCN_x and PSCN_x animals was less than 50% of those nuclei. There was no damage to the ventromedial hypothalamus (VMH), lateral hypothalamus (LH) or to sleep-related areas such as the ventrolateral preoptic nucleus (VLPO) or the dorsomedial hypothalamus (DMH). Damage to the surrounding areas was not consistent among animals and there was no single area that correlated with SCN rescue of memory.

None of the PSCN_x animals recovered memory function, so we could not locate a critical subregion within the SCN that was necessary for NOR and SA performance. As for the tissue damage: in 5 animals, 50-60% of the SCN was destroyed. In 3 of those animals, the damage was limited to the rostral/mid SCN along its dorsoventral extent, and to the caudal SCN along the dorsoventral plane in the other 2 hamsters. The remaining 4 animals had 10-20% of SCN tissue destroyed and in all of those hamsters, the damage was in the dorsal mid-SCN.

The optic chiasm and tracts were observed to be intact during brain removal and tissue sectioning. The sectioning process would occasionally damage the optic chiasm in lesioned animals, as the lesion destroys gray matter structurally connecting the chiasm to the brain. In Siberian hamsters, the chiasm is quite thick rostrally and thins out caudally (Fig. S6). Some damage to the retinohypothalamic tract is inevitable, but the optic tracts were not damaged, thus, there is no anatomical evidence that our lesioned animals were visually impaired. Nevertheless, without performing visual tests, we cannot rule out the possibility that modest visual impairments in SCN_x animals might strengthen other sensory modalities such as olfaction which might lead to improved performance on the memory tests.

As few as 10% of SCN neurons remaining after a lesion are sufficient to drive body temperature rhythms in rats, Siberian hamsters, squirrels monkeys, and ground squirrels (31-34). The location of these residual SCN neurons has not been consistent across animals or across studies, thus, the same may be true for SCN control of NOR and SA performance.

Behavior of Hamsters During the NOR Task:

Examples of object exploration during the test phase of the NOR task are provided for experiment 2 during the ENT (Movie S1: NOR Test Phase: ENT), DPS (Movie S2: NOR Test Phase: DPS), and SCNx (Movie S3: NOR Test Phase: SCNx) conditions of an individual animal. In each movie, the novel object is positioned to the right side of the arena.

References:

1. I. R. Rouch, P. Wild, D. Ansiau, J-C. Marqué, Shiftwork experience, age and cognitive performance. *Ergonomics* **48**, 1282-1293 (2005).
2. G. J. Tranah, T. Blackwell, K. L. Stone, S. Ancoli-Israel, M. L. Paudel, *et al.*, Circadian activity rhythms and risk incident dementia and mild cognitive impairment in older women. *Ann. Neurol.* **70**, 722-732 (2011).
3. G. E. S. Covell, P. S. Dhawan, J. K. L. Iannotti, C. R. Hoffman-Snyder, K. E. Wellik, *et al.*, Disrupted daytime activity and altered sleep-wake patterns may predict transition to mild cognitive impairment or dementia. *The Neurologist* **18**, 426-429 (2012).
4. E. J. W. van Someren, E. E. O. Hagebeuk, C. Lijzenga, P. Scheltens, S. E. J. A. de Rooij, *et al.*, Circadian rest-activity disturbances in Alzheimer's disease. *Biol. Psychiatry* **40**, 259-270 (1996).
5. A. N. Coogan, B. Schutová, S. Husung, K. Furczyk, B. T. Baune, *et al.*, The circadian system in Alzheimer's disease: disturbances, mechanisms, and opportunities. *Biol. Psychiatry* **74**, 333-339 (2013).
6. Y-H. Wu, D. F. Swaab, Disturbance and strategies for reactivation of the circadian rhythm system in aging and Alzheimer's disease, *Sleep Med.* **8**, 623-636 (2007).
7. E. A. Van Der Zee, R. Havekes, R. P. Barf, R. A. Hut, I. M. Nijholt, *et al.*, Circadian time-place learning in mice depends on *cry* genes. *Curr. Biol.* **18**, 844-848 (2008).
8. C. Mulder, E. A. Van Der Zee, R. A. Hut, M. P. Gerkema, Time-place learning and memory persist in mice lacking functional *Per1* and *Per2* clock genes. *J. Biol. Rhythms* **28**, 367-379 (2013).
9. S. Wardlaw, T. H. Phan, A. Saraf, X. Chen, D. R. Storm, Genetic disruption of the core circadian clock impairs hippocampus-dependent memory. *Learn. Mem.* **21**, 417-423 (2014).
10. E. A. Yu, D. R. Weaver, Disrupting the circadian clock: gene-specific effects on aging, cancer, and other phenotypes. *Aging* **3**, 479-493 (2011).
11. F. Stephan, N. S. Kovacevic, Multiple retention deficits in passive avoidance in rats is eliminated by suprachiasmatic lesions. *Behav. Biol.* **22**, 456-462 (1978).
12. R. E. Mistlberger, M. H. de Groot, J. M. Bossert, E. G. Marchant, Discrimination of circadian phase in intact and suprachiasmatic nuclei-ablated rats. *Brain Res.* **739**, 12-18 (1996).
13. S. W. Cain, M. R. Ralph, Circadian modulation of conditioned place avoidance in hamsters does not require the suprachiasmatic nucleus, *Neurobiol. Learn. Mem.* **1**, 81-84 (2009).

14. T. X. Phan, G. C. Chan, C. B. Sindreu, K. L. Eckel-Mahan, D. R. Storm, The diurnal oscillation of MAP (mitogen-activated protein) kinase and adenylyl cyclase activities in the hippocampus depend on the suprachiasmatic nucleus, *J. Neurosci.* **31**, 10640-10647 (2011).
15. B. L. Smarr, K. J. Jennings, J. R. Driscoll, L. J. Kriegsfeld, A time to remember: the role of circadian clocks in learning and memory, *Behav. Neurosci.* **128**, 283-303 (2014).
16. T. DeBoer, Investigating sleep homeostasis using an unusual instability, *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **287**, R8-R9 (2004).
17. S. Steinlechner, A. Stieglitz, T. Ruf, Djungarian hamsters: a species with a labile circadian pacemaker? Arrhythmicity under a light-dark cycle induced by short light pulses, *J. Biol. Rhythms* **17**, 248-258 (2002).
18. B. P. Grone, D. Chang, P. Bourgin, V. Cao, R. D. Fernald, *et al.*, Acute light exposure suppresses circadian rhythms in clock gene expression, *J. Biol Rhythms* **26**, 78-81 (2011).
19. N. F. Ruby, C. E. Hwang, C. Wessells, F. Fernandez, P. Zhang, *et al.*, Hippocampal-dependent learning requires a functional circadian system, *Proc. Natl. Acad. Sci. USA* **7**, 15593-15598 (2008).
20. N. F. Ruby, F. Fernandez, A. Garrett, J. Klima, P. Zhang, *et al.*, Spatial memory and long-term object recognition are impaired by circadian arrhythmia and restored by the GABA_A antagonist pentylentetrazole, *PLoS One* **8**, e72433. Doi:10.1371/journal.pone.0072433.
21. R. M. Deacon, J. N. Rawlins, T-maze alternation in the rodent, *Nat. Protoc.* **1**, 7-12 (2006).
22. Materials and methods are available as supplementary materials on *Science Online*.
23. C. H. Ko, R. J. McDonald, M. R. Ralph, The suprachiasmatic nucleus is not required for temporal gating of performance on a reward-based learning and memory task, *Biol. Rhythms Res.* **34**, 177-192 (2003).
24. N. F. Ruby, J. Dark, D. E. Burns, H. C. Heller, I. Zucker, The suprachiasmatic nucleus is essential for circadian body temperature rhythms in hibernating ground squirrels, *J. Neurosci.* **22**, 357-364 (2002).
25. D. G. Harper, E. G. Stopa, V. Kuo-Leblanc, A. C. McKee, K. Asayama, *et al.*, Dorosmedial SCN neuronal subpopulations subserve different functions in human dementia, *Brain* **131**, 1609-1617 (2008).
26. F. W. Turek, Staying off the dance floor: when no rhythm is better than bad rhythm, *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **294**, R1672-R1674 (2008).
27. A. T. Winfree, *The Geometry of Biological Time* (Springer-Verlag, New York, 1980).

28. J. E. Larkin, T. Yokogawa, H. C. Heller, P. Franken, N. F. Ruby, Homeostatic regulation of sleep in arrhythmic Siberian hamsters. *Am. J. Physiol.* **287**, R104-R111 (2004).
29. L. Trachsel, D. M. Edgar, W. F. Seidel, H. C. Heller, W. C. Dement, Sleep homeostasis in suprachiasmatic nuclei-lesioned rats: effects of sleep deprivation and triazolam administration. *Brain Res.* **589**, 253-261 (1992).
30. A. Easton, P. Meerlo, B. Bergmann, F. W. Turek, The suprachiasmatic nucleus regulates sleep timing and amount in mice. *Sleep* **27**, 1307-1318 (2004).
31. E. Satinoff, R. A. Prosser, Suprachiasmatic nuclear lesions eliminate circadian rhythms of drinking and activity, but not of body temperature, in male rats. *J. Biol. Rhythms* **3**, 1-22 (1988).
32. N. F. Ruby, I. Zucker, Daily torpor in the absence of the suprachiasmatic nucleus. *Am. J. Physiol.* **263**, R353-R362 (1989).
33. D. M. Edgar, W. C. Dement, C. A. Fuller, Effect of SCN lesions on sleep in squirrel monkeys: evidence for opponent processes in sleep-wake regulation. *J. Neurosci.* **13**, 1065-1079 (1993).
34. N. F. Ruby, J. Dark, H. Craig Heller, I. Zucker, Ablation of the suprachiasmatic nucleus alters timing of hibernation in ground squirrels. *Proc. Natl. Acad. Sci.* **93**, 9864-9868 (1996).

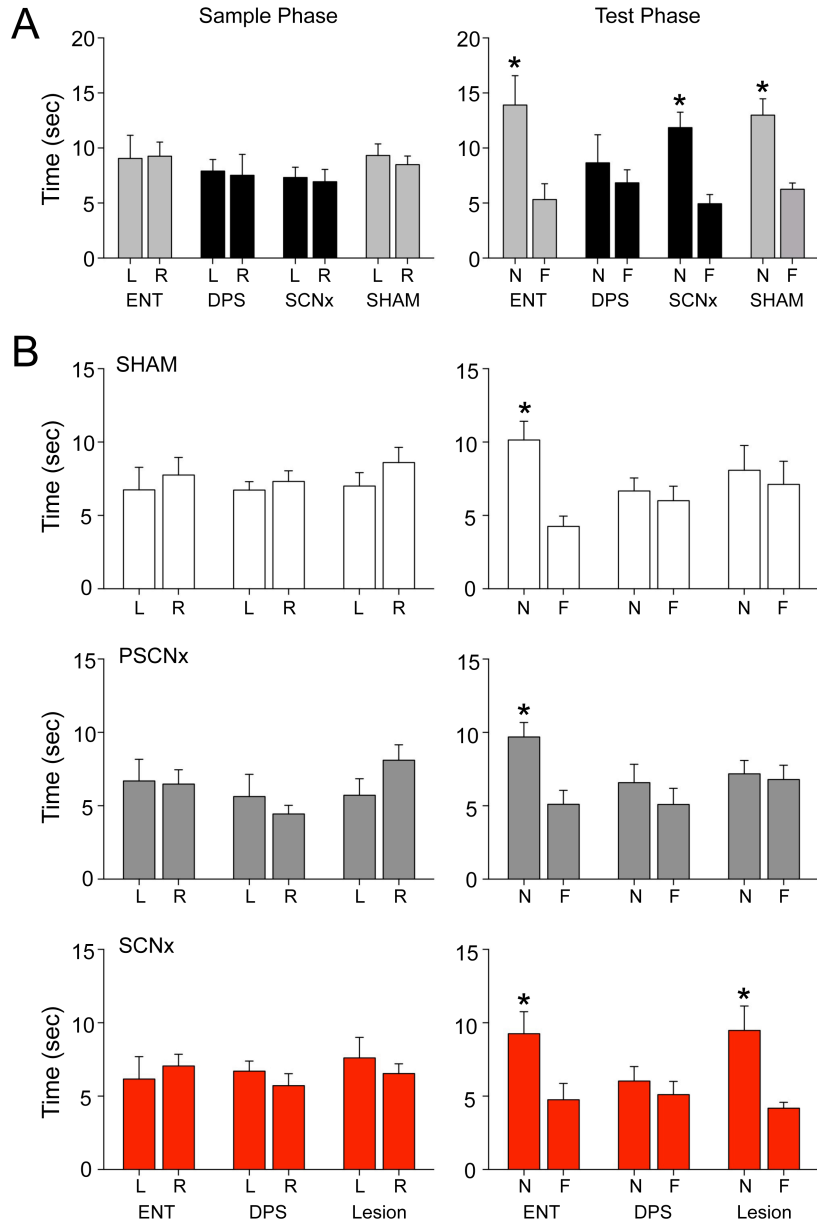


Figure S1. Exploration times during the sample (left panels) and test (right panels) phases of the NOR task in experiments 1 (A) and 2 (B). See figure legends 1 and 2 for abbreviations. Objects in the NOR arena are placed in the left (L) and right (R) rear corners of the arena. In the test phase, objects are identified as familiar (F) or novel (N). * indicates that time spent with N was significantly greater than time spent with F (paired t-test, $0.001 < P < 0.01$).

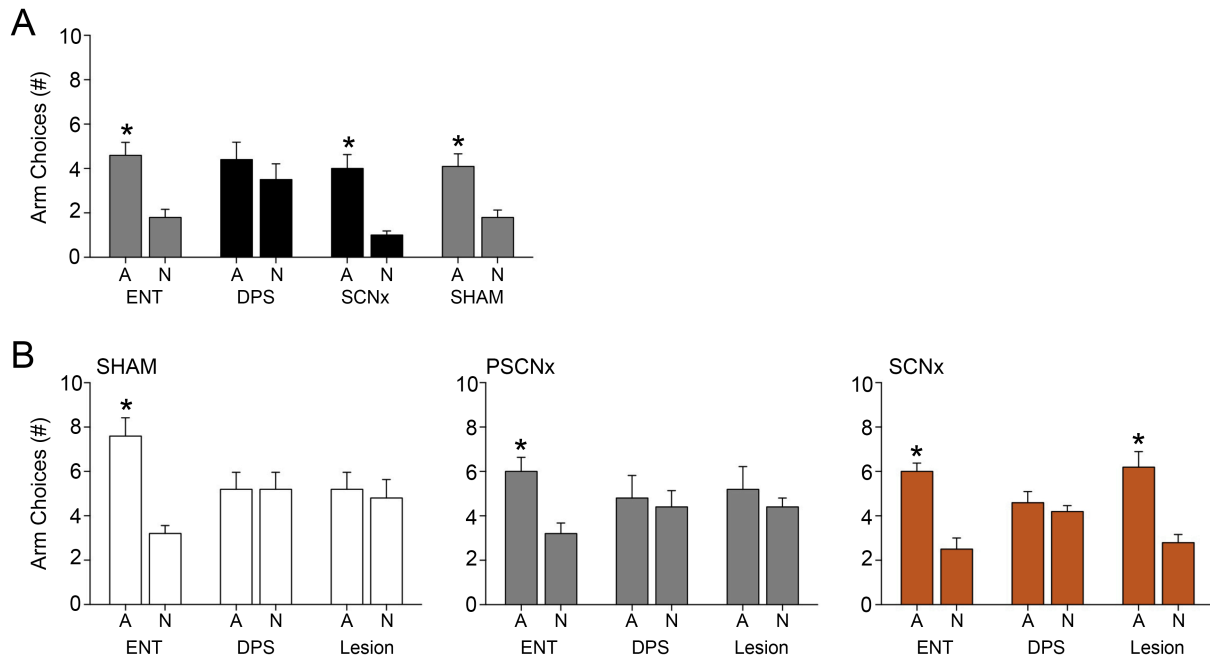


Figure S2. Exploration defined as the number of arm entries during the SA test in experiments 1 (A) and 2 (B). Each entry into the left or right arm of the T-maze was either an alternation “A” or a nonalternation “N”. * indicates significantly more alternations than nonalternations (paired t-test, $0.001 < P < 0.01$).

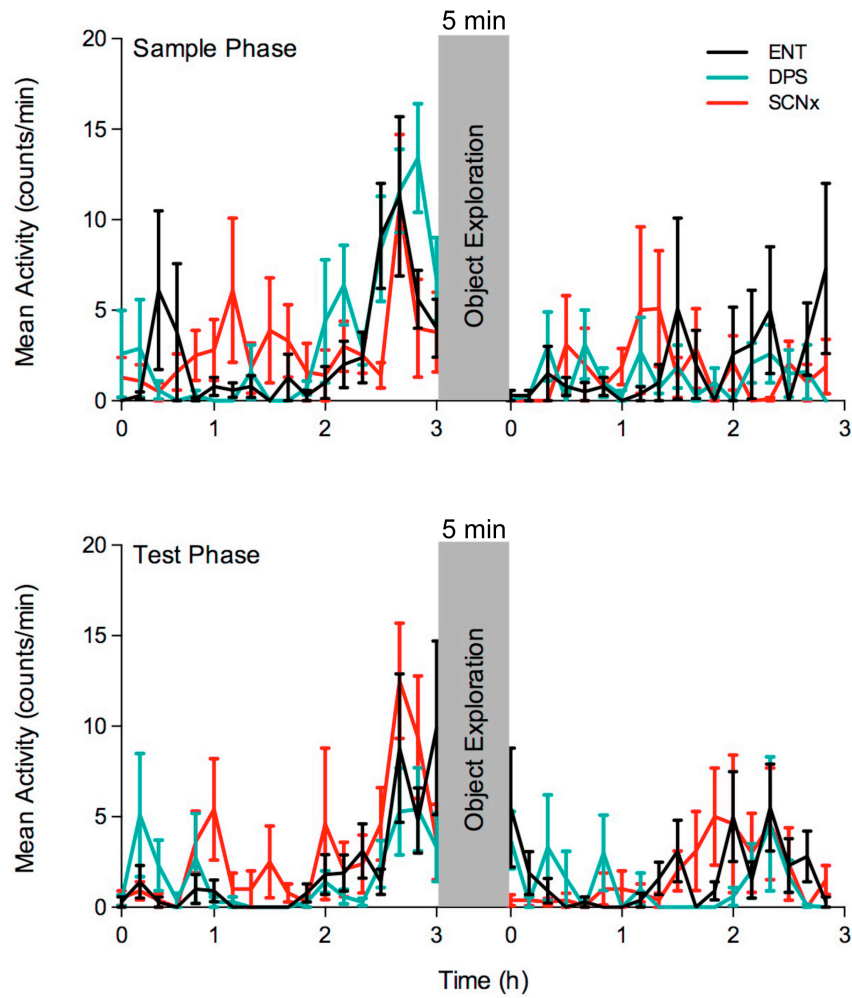


Figure S3. Locomotor activity in home cages before and after object exploration in the NOR task in experiment 2 plotted in 10-min intervals. See figure legend 3 for abbreviations. All animals were awake and vigilant prior to object exploration and rested for 60-90 min afterwards. Note that there are no differences in activity across all three conditions. See text for statistical analysis.

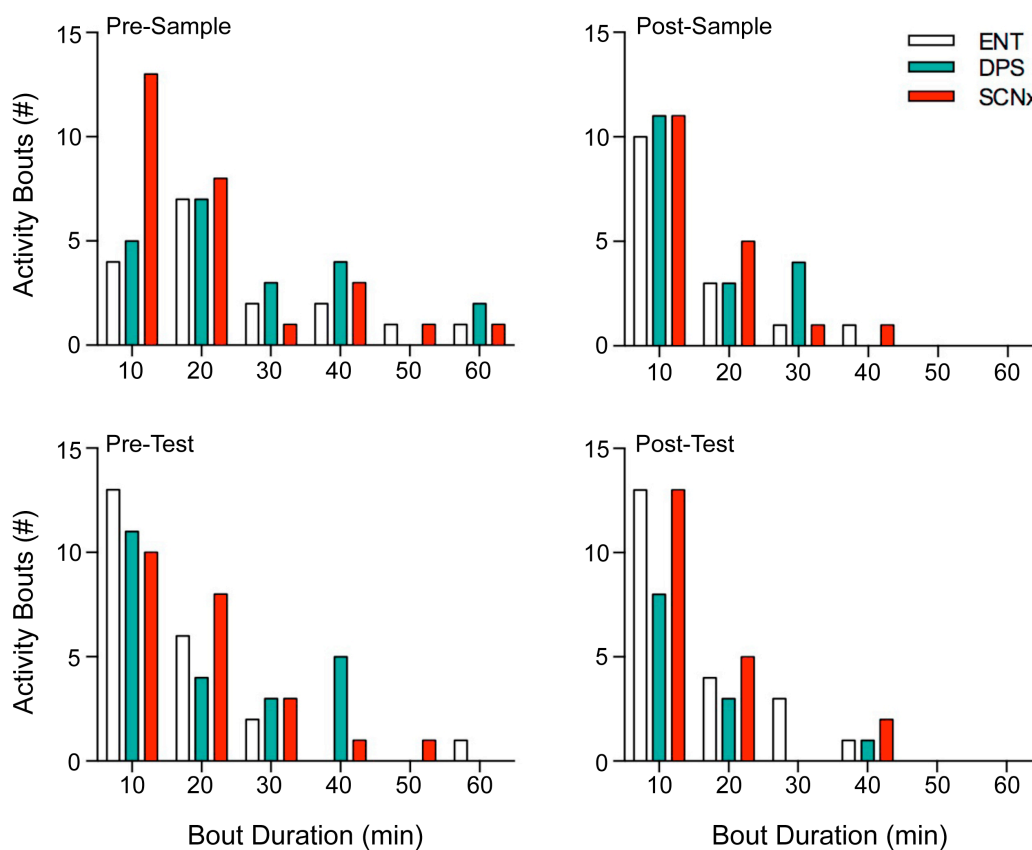


Figure S4. Frequency histograms of activity bout ranging in duration from 10-60 min taken from the 3-h periods before and after the sample and test phases of the NOR task. Most activity bouts were 10-20 min in duration and there were no differences across the three conditions. See text for statistical analyses.

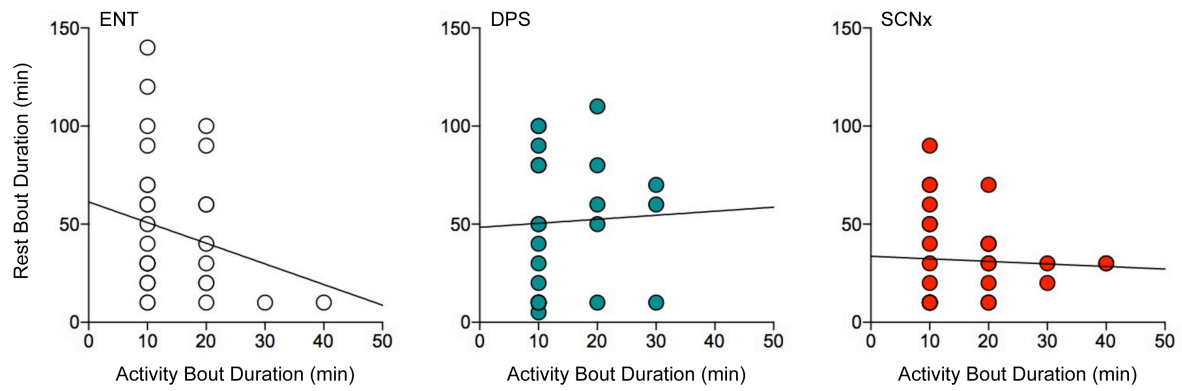


Figure S5. Relationship between activity bout duration and subsequent rest bout duration during the 3-h before and after object exploration in the NOR task. No significant correlations were found in any of the three conditions. See text for statistical analyses.

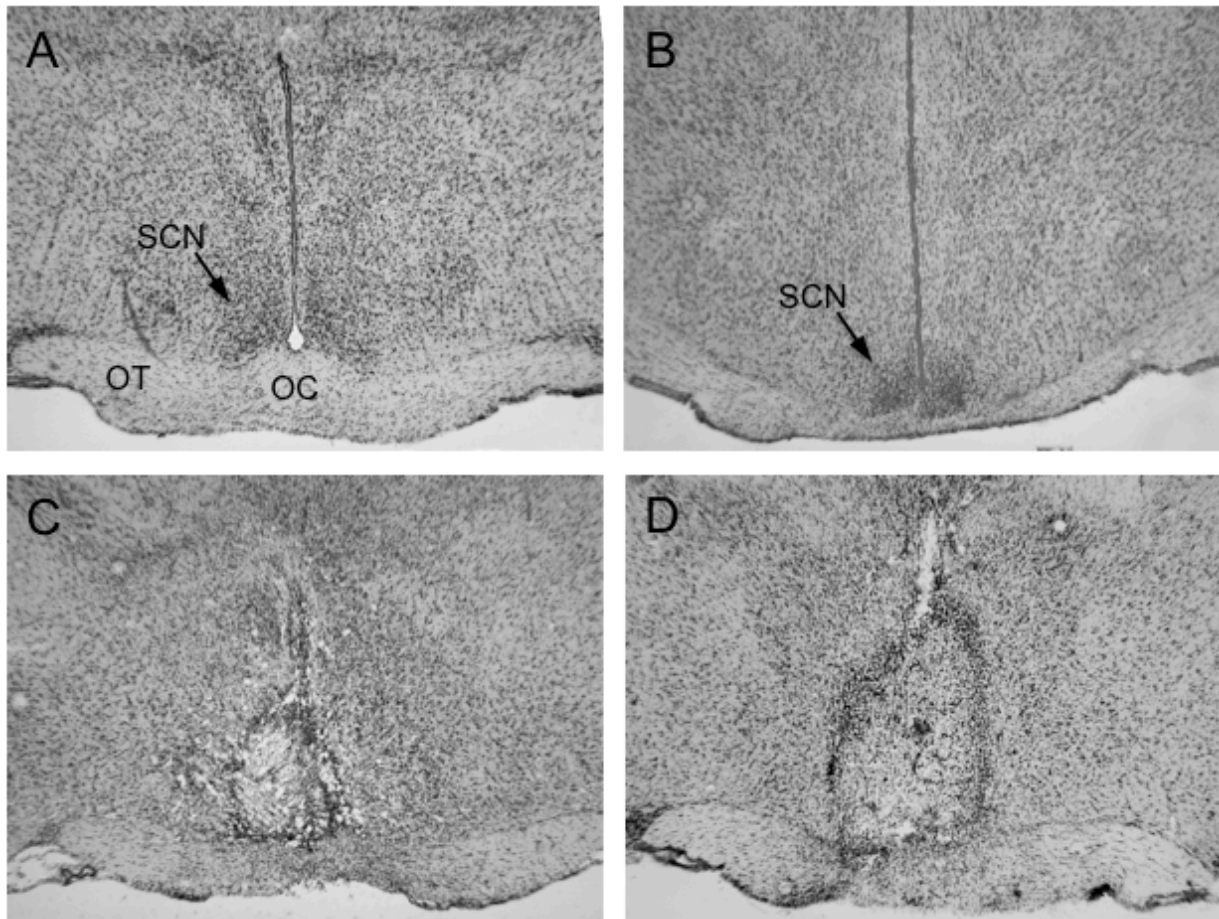


Figure S6. Tissue sections through the SCN of intact (A, B) and lesioned (C, D) hamsters. The optic chiasm (OC) is quite thick rostrally (A) and thins out caudally (B) as seen in sections from the same animal. Lesions did not extend very far beyond the SCN boundaries, nor did they damage the optic tracts (OT) as shown in two different animals (C, D).