Aberrant light directly impairs mood and learning through melanopsin-expressing neurons

LeGates, TA et. al.

Supplementary Text:

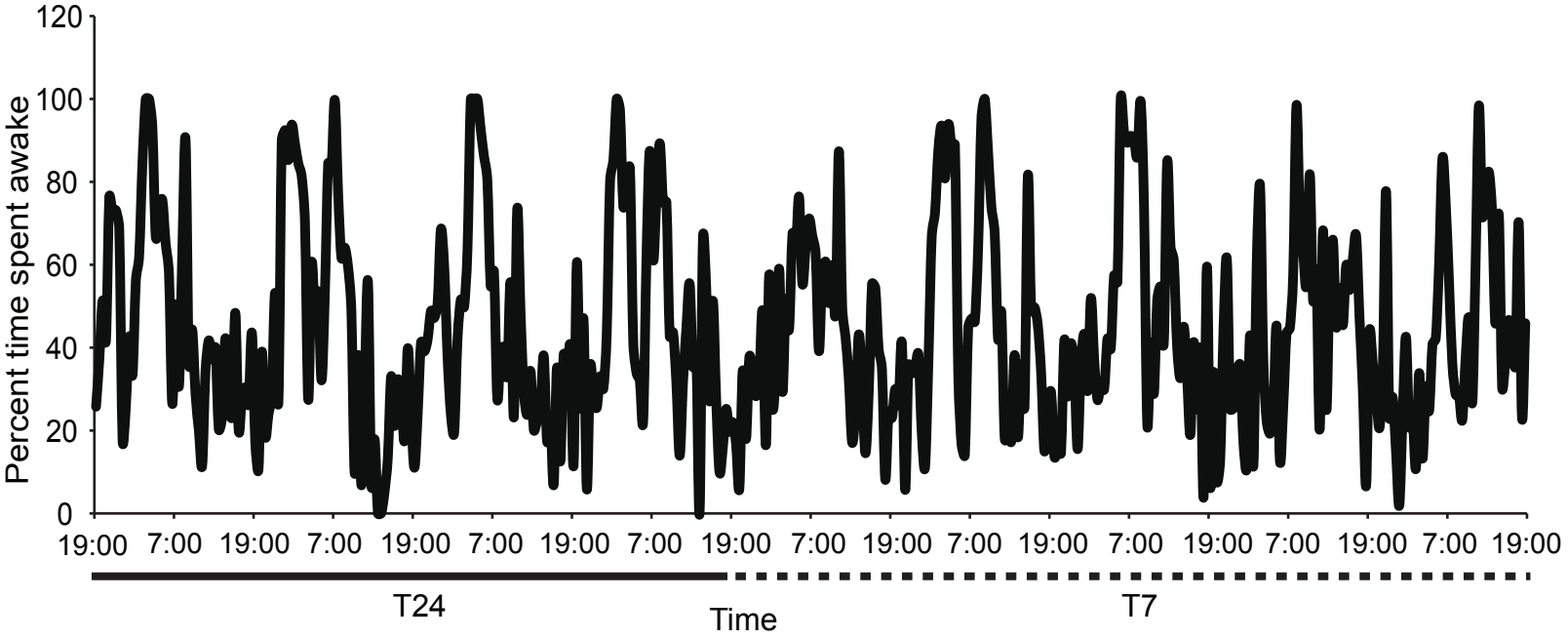
Wheel-running activity has secondary effects on behavior
Previous studies utilized wheel-running activity to assay the influence the T7 cycles on circadian rhythms[1, 2]. Since wheel running behavior is known to impact circadian photoentrainment, mood, and learning[3-7], we assayed circadian light responses in the T7 cycle in the absence of a wheel by measuring core body temperature rhythms (Figure S1). All temperature rhythms in mice under the T7 cycle showed period lengths slightly longer than 24 hours (Figure S1).

T7 mice locate the platform using non-spatial strategy
In addition to the spatial strategy mentioned in the manuscript, a non-spatial escape strategy relies on circling the pool at the correct distance from the wall (platform annulus) until finding the platform[8]. To determine if mice placed in the T7 or the T24 cycle used this non-spatial strategy, we determined the percent of time that mice spent swimming in the platform annulus during the probe trial. We found that both the T7 and the T24 mice spent significantly more time in this area as compared to chance encounter of the quadrants (T24: 40.83±1.51%, n=9, $p<0.0001$; T7: 39.83±2.51%, n=9, $p<0.0001$). This indicates that the T7 mice relied on the non-spatial strategy to locate the platform, whereas the T24 mice paired the non-spatial strategy with a cue-based approach.

Treatment with the antidepressant desipramine restores hippocampal-dependent learning
In the manuscript, we show that chronic treatment with the antidepressant fluoxetine was able to decrease depression related behavior and rescue the learning deficit observed in mice housed in the T7 cycle (Figure 3). We sought to investigate whether treatment with an alternative antidepressant could rescue the learning deficits observed in T7 mice. Mice received 2 intraperitoneal injections (24- and 1-hour before testing) of 16 mg/kg desipramine, a tricyclic antidepressant[23], prior to the Novel Object Recognition test. We found that desipramine administration restored learning in the T7 cycle exposed mice (Figure S4). Mice housed in the T24 cycle treated with desipramine showed decreased time spent with the novel object in agreement with previous reports that desipramine induces learning defects in mice placed under normal light environment[24]. These results show that the detrimental effects of aberrant light on learning could be alleviated with antidepressant administration.

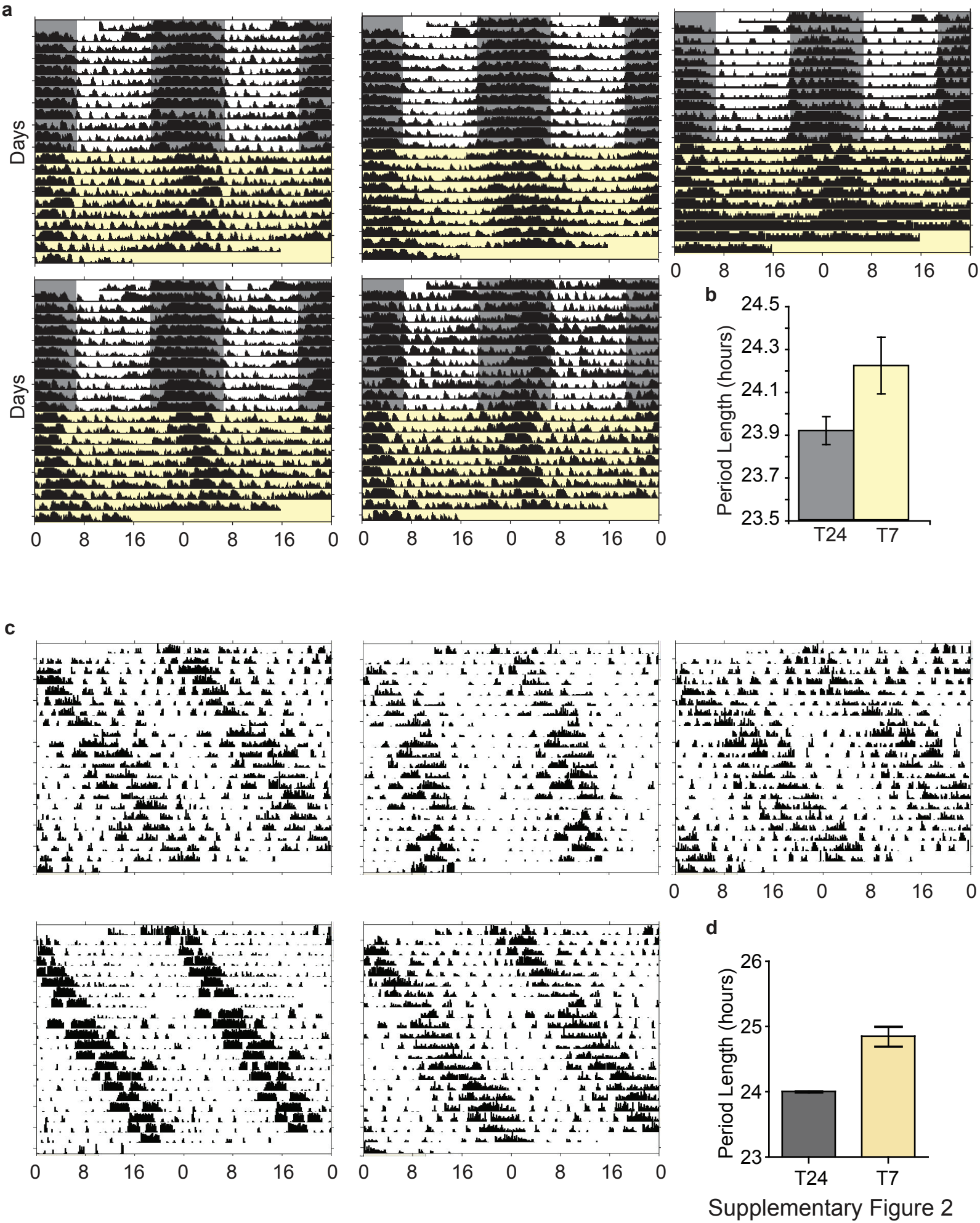
Timing of light administration is crucial for physiology
Bright light is used as a treatment for seasonal depression in humans[9, 10], whereas, it has been shown to have anxiogenic effects in mice[11, 12]. Therefore, when determining the effects of light on behavior, a common assumption is that light is beneficial to diurnal organisms and aversive to nocturnal animals. However, rats placed in constant darkness show increased depression-like behavior[13], which may be due to lack of beneficial light stimulations in this nocturnal animal. Recent studies in Drosophila indicate that even in the same organism, preference or avoidance of light changes during the animals' lifespan[14]. Furthermore, light and dark influence sleep and alertness in both nocturnal and

diurnal animals. Therefore, the direct influence of light on physiology could be disruptive or beneficial depending on the timing of the light input.



Supplementary Figure 1

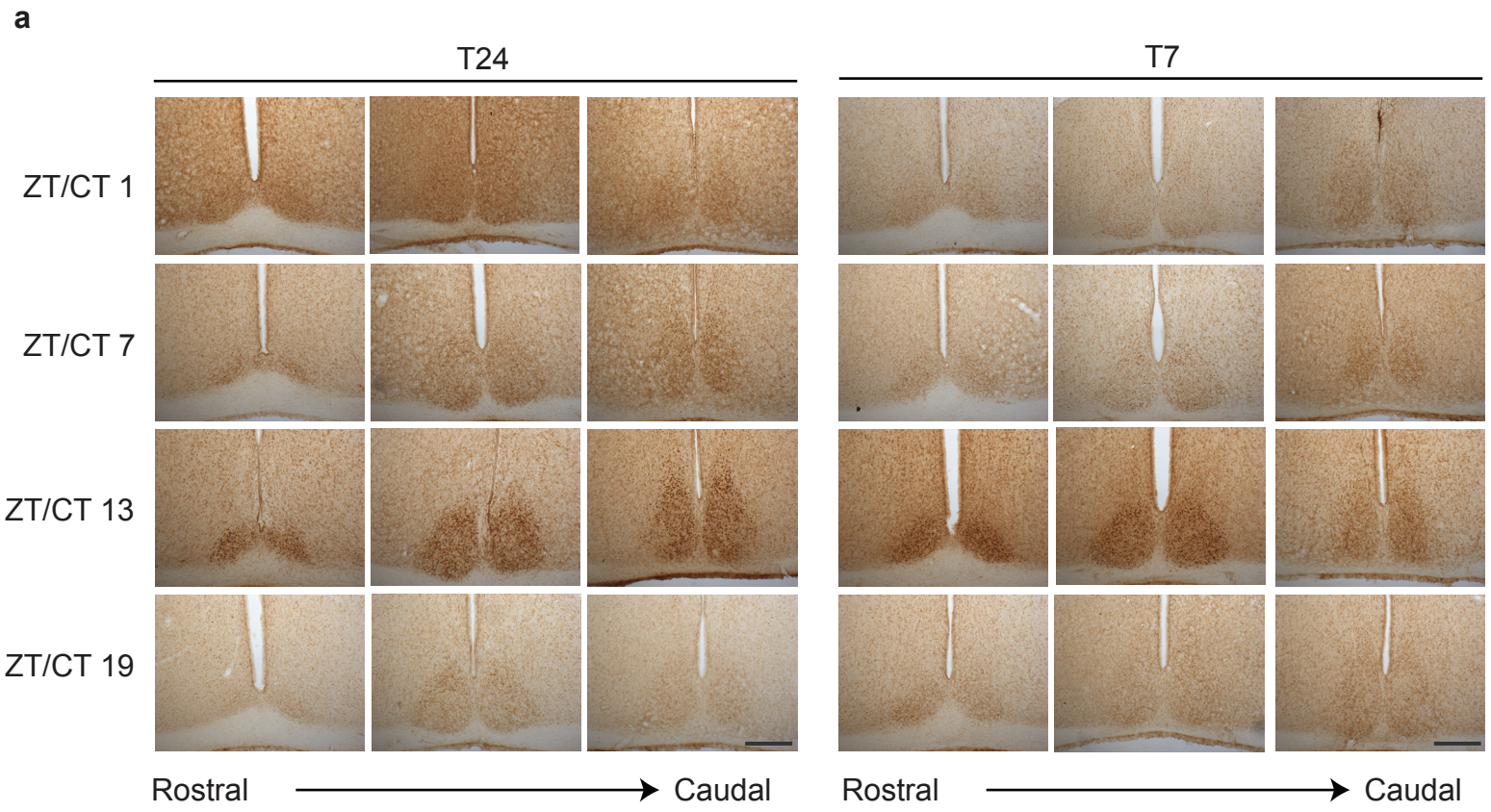
Supplementary Figure 1: The T7 light cycle does not disrupt sleep distribution
The average wakefulness in 30-minute bins for the mice is plotted over 9 days. Mice were housed in the T24 cycle on Days 1-4 (solid line along the x-axis) and in the T7 cycle from Days 5-9 (dotted line along the x-axis). There was a disruption in the sleep pattern on Day 5, which returned to normal by Day 6.

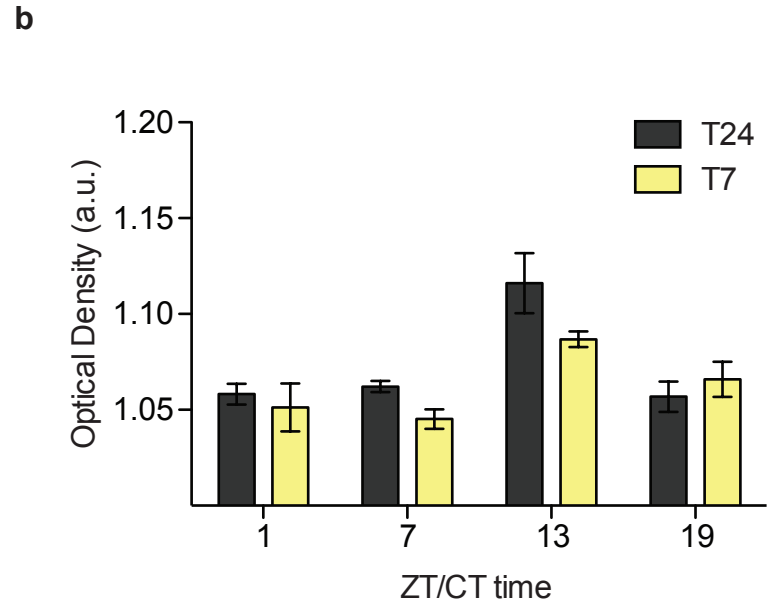


Supplementary Figure 2

Supplementary Figure 2: The T7 light cycle increases the period length of temperature and general activity rhythms

A. Actograms of core body temperature rhythms in T24 (grey and white background) and T7 (yellow background) light cycle. **B.** Mice housed in the T7 light cycle showed an increased period 24.20±0.13 (n=5) as measured by core body temperature, whereas, mice housed in the T24 light cycle showed a period of 23.92 ± 0.07 (n=6). **C.** Actograms of general activity rhythms of mice under the T7 light cycle. **D.** Mice housed in the T7 light cycle showed an increased period of 24.85 ± 0.15 (n=8) as compared to mice housed in the T24 light cycle which showed a period of 24.01 ± 0.01 (n=7). Error bar indicates SEM.





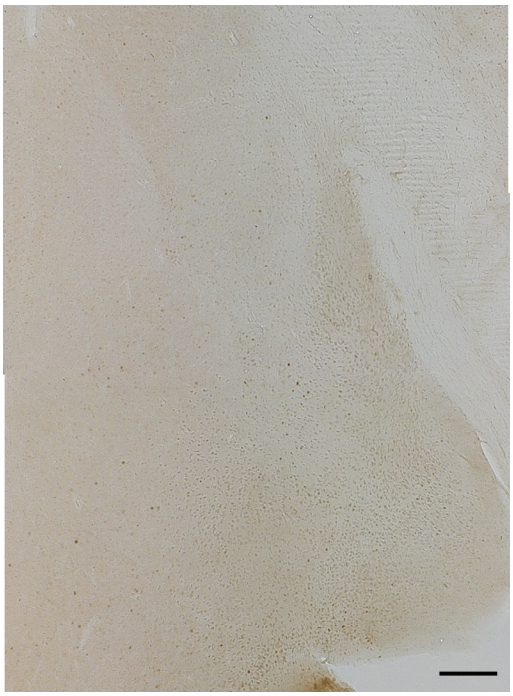
Supplementary Figure 3

Supplementary Figure 3: Rhythmic PERIOD2 expression remains intact in mice housed in the T7 light cycle

A. Representative images from rostral to caudal portion of the SCN show that levels of PER2 were rhythmic in mice housed in the T24 and T7 light cycles peaking at ~ZT/CT13. Scale bar, 200μm. Additionally, localization of PER2 positive cells was similar between the two groups. Brown puncta represent PER2 positive cells. Images taken at 10x magnification. **B.** Quantification of SCN PER2 expression (n=7-8 per group at each time point, $p_{time}<0.0001$).

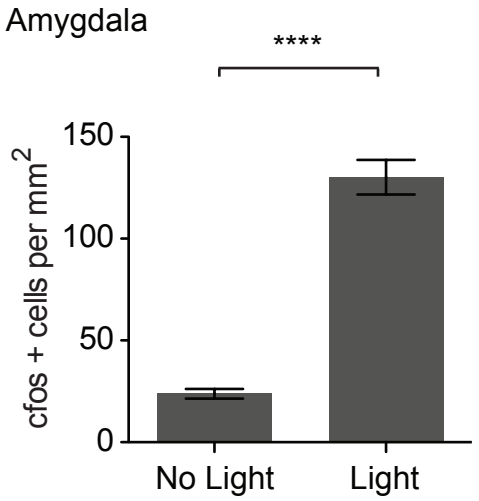
a

Amygdala



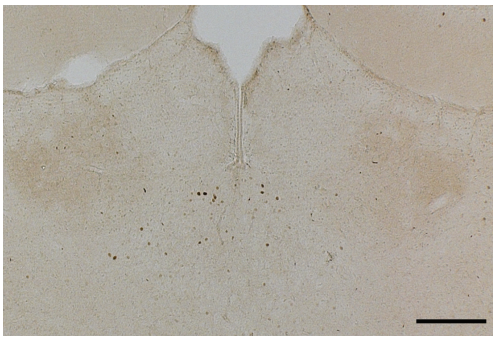
No Light

Light

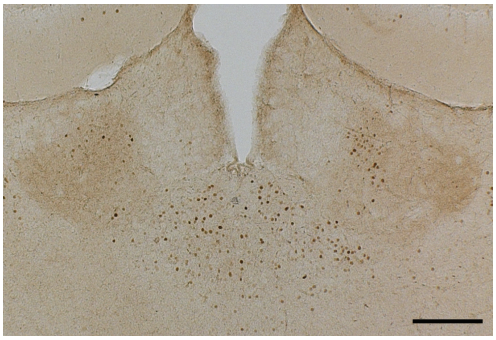


b

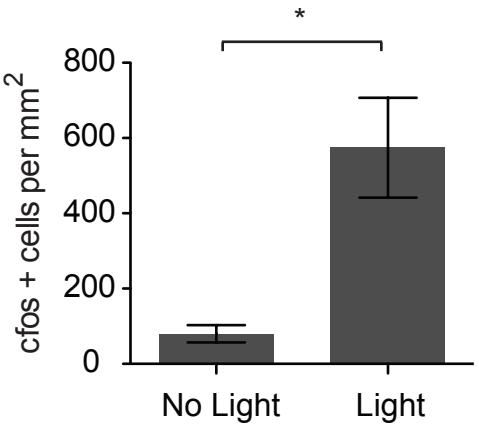
Habenula



No Light

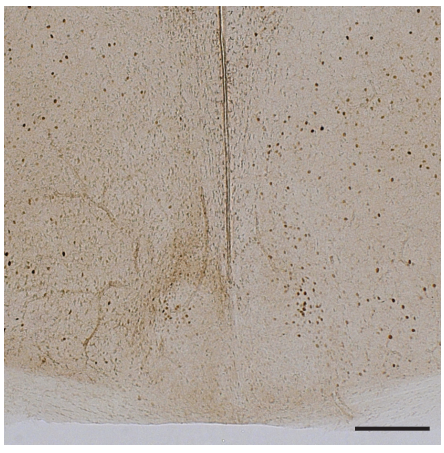


Light

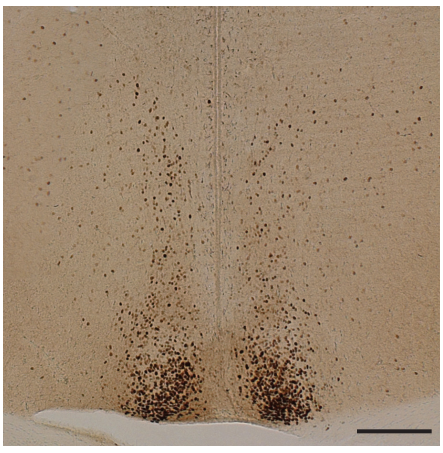


c

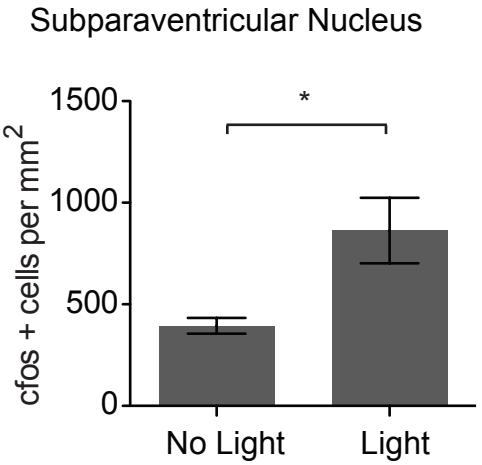
Subparaventricular nucleus and Suprachiasmatic nucleus



No Light



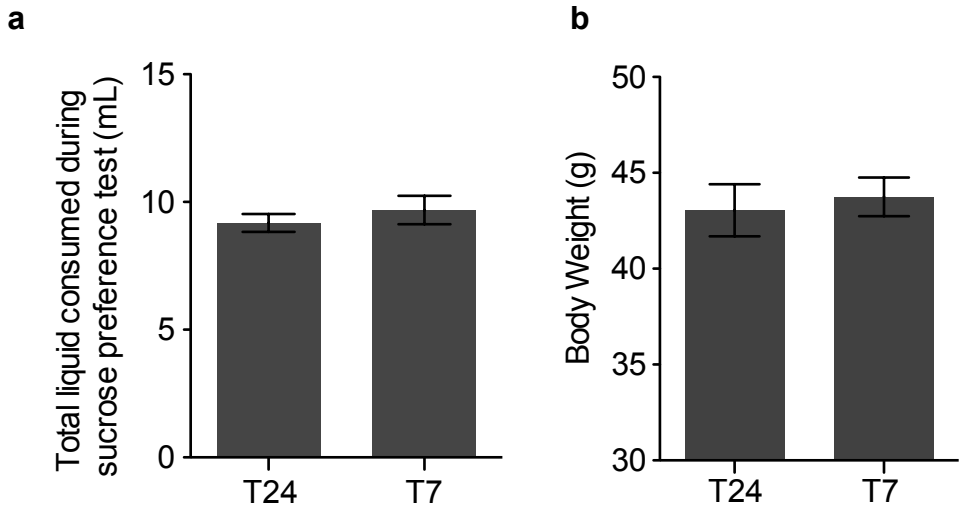
Light



Supplementary Figure 4

Supplementary Figure 4: Light at night induces c-fos expression in the amygdala, habenula, and subparaventricular zone

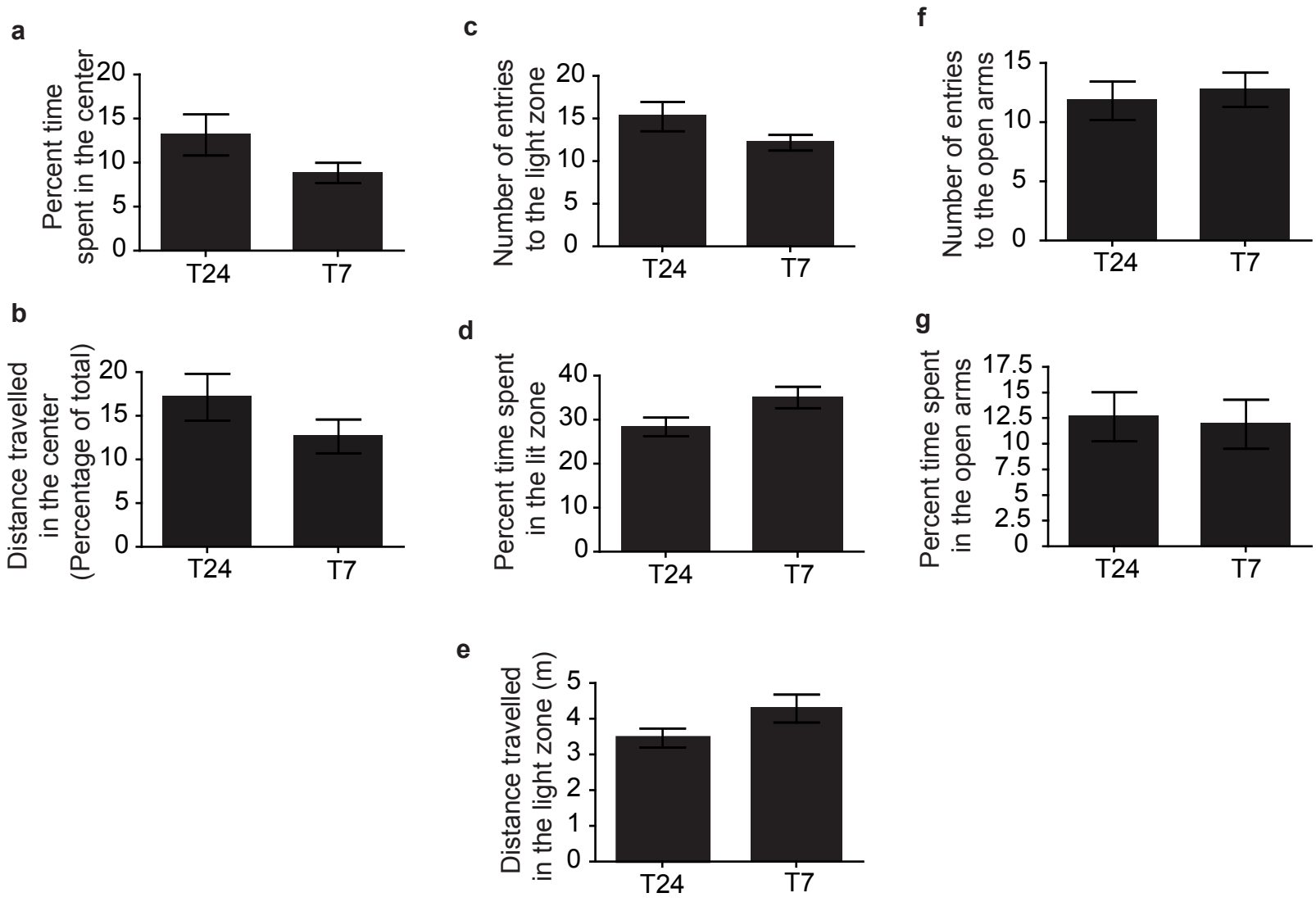
A. (Left) Representative images from the amygdala of a mouse in darkness and a mouse that received a 10-minute light pulse at approximately ZT14. Scale bar, 200µm. (Right) Quantification shows increased c-fos positive cells in the amygdala in response to a light pulse (n=3-5 per group, unpaired t-test $p<0.0001$). **B.** (Left) Representative images from the habenula of a mouse in darkness and a mouse that received a 10-minute light pulse at approximately ZT14. Scale bar, 200µm. (Right) Quantification shows increased c-fos positive cells in the lateral habenula in response to a light pulse (n=3-5 per group, unpaired t-test $p=0.018$). **C.** (Left) Representative images from the subparaventricular nucleus of a mouse in darkness and a mouse that received a 10-minute light pulse at approximately ZT14. Scale bar, 200µm. (Right) Quantification shows increased c-fos positive cells in the subparaventricular nucleus in response to a light pulse (n=3-5 per group, unpaired t-test $p=0.015$). Brown puncta represent c-fos positive cells. All images were taken at 5x magnification. **** indicates $p<0.0001$, * indicates $p< 0.05$. Error bar indicates SEM.



Supplementary Figure 5

Supplementary Figure 5: T7 light cycles does not alter total liquid consumption or body weight

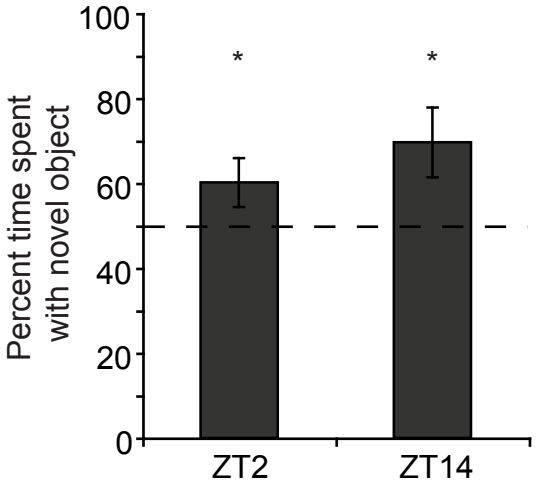
A. Total amount of liquid consumed during the sucrose preference test did not differ between mice housed in the T24 and T7 light cycles (n=16,15, unpaired t-test p=0.44). **B.** Body weight was similar between mice housed in the T24 and T7 cycle (n=20 per group, unpaired t-test p=0.68). Error bar indicates SEM.



Supplementary Figure 6

Supplementary Figure 6: T7 light cycle does not cause an increase in anxiety-related behaviors

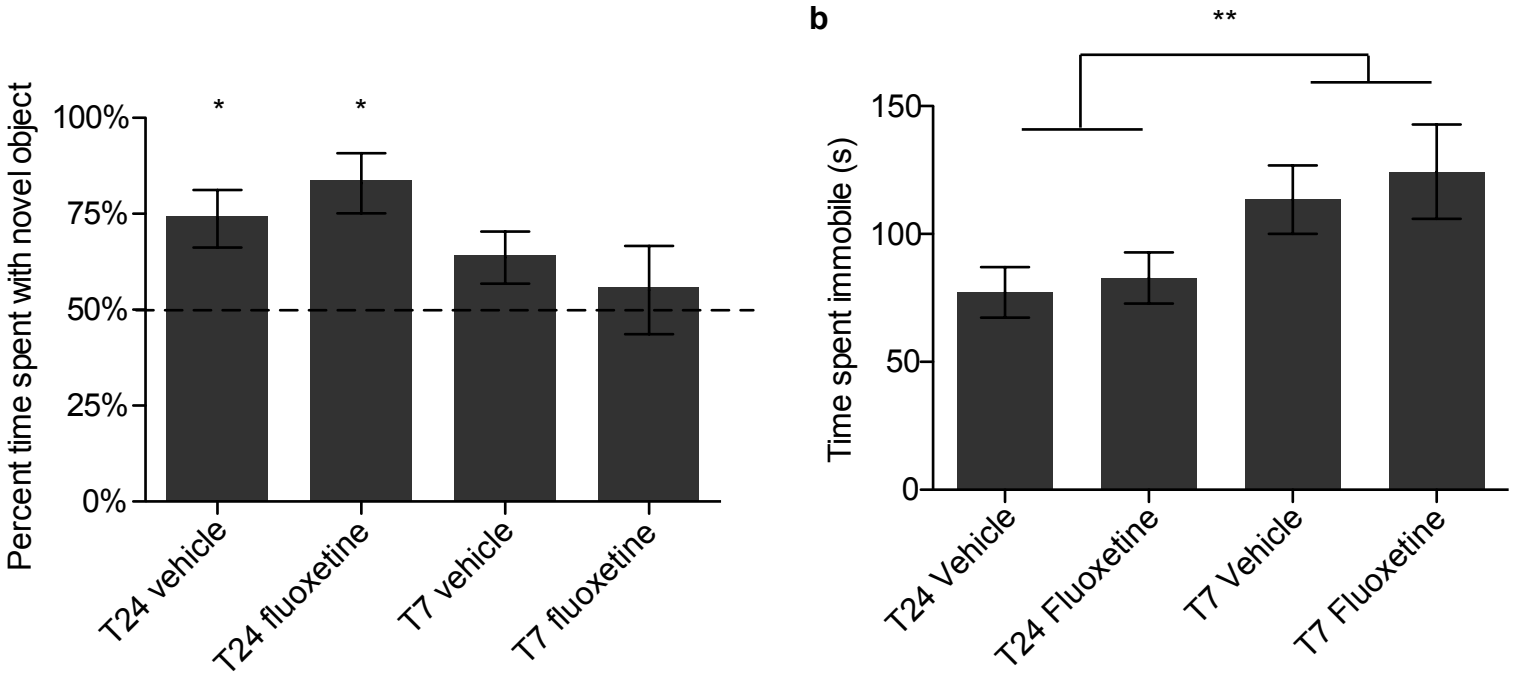
A and B. Mice housed in the T7 cycle showed no significant difference in anxiety-related measures in the open field as measured by percent time (A) (n=12 per group, p=0.1137) or distance traveled (B) (n=12 per group, p=0.1890) in the center of the field. **C-E.** Mice housed in the T7 and T24 light cycles showed no significant differences in anxiety-related behavior in the light dark box as measured by number of entries to the light zone (C) (n=12,11, p=0.19), percent time in the light zone (D) (n=12,11, p=0.05), or distance travelled in the light zone (E) (n=12,11, p=0.09). **F and G.** Mice housed in the T7 and T24 light cycles showed no significant differences in anxiety-related behavior in the elevated plus maze as measured by number of entries to the open arms (F) (n=12 per group, p=0.68) or percent time spent in the open arms (G) (n=12 per group, p=0.83). Error bar indicates SEM.



Supplementary Figure 7

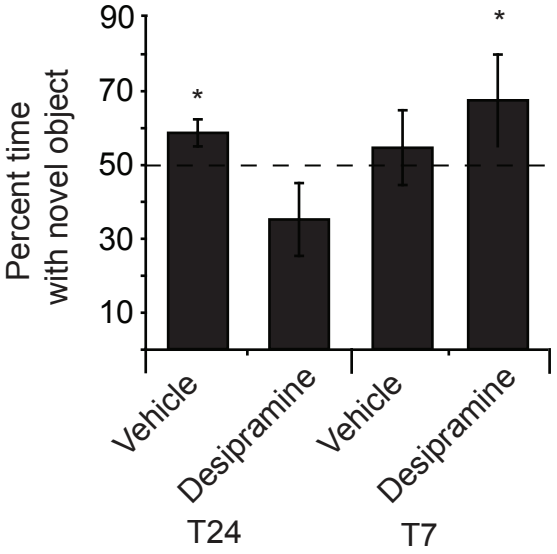
Supplementary Figure 7: Hippocampal learning is observed both during day and night

In the novel object recognition task, mice housed in ZT2 and ZT14 both spent more time with the novel object (ZT2: n=12, $p<0.001$; ZT14: n=11, $p=0.04$). No significant difference was found between the two groups ($p=0.34$). Error bar indicates SEM.



Supplementary Figure 8

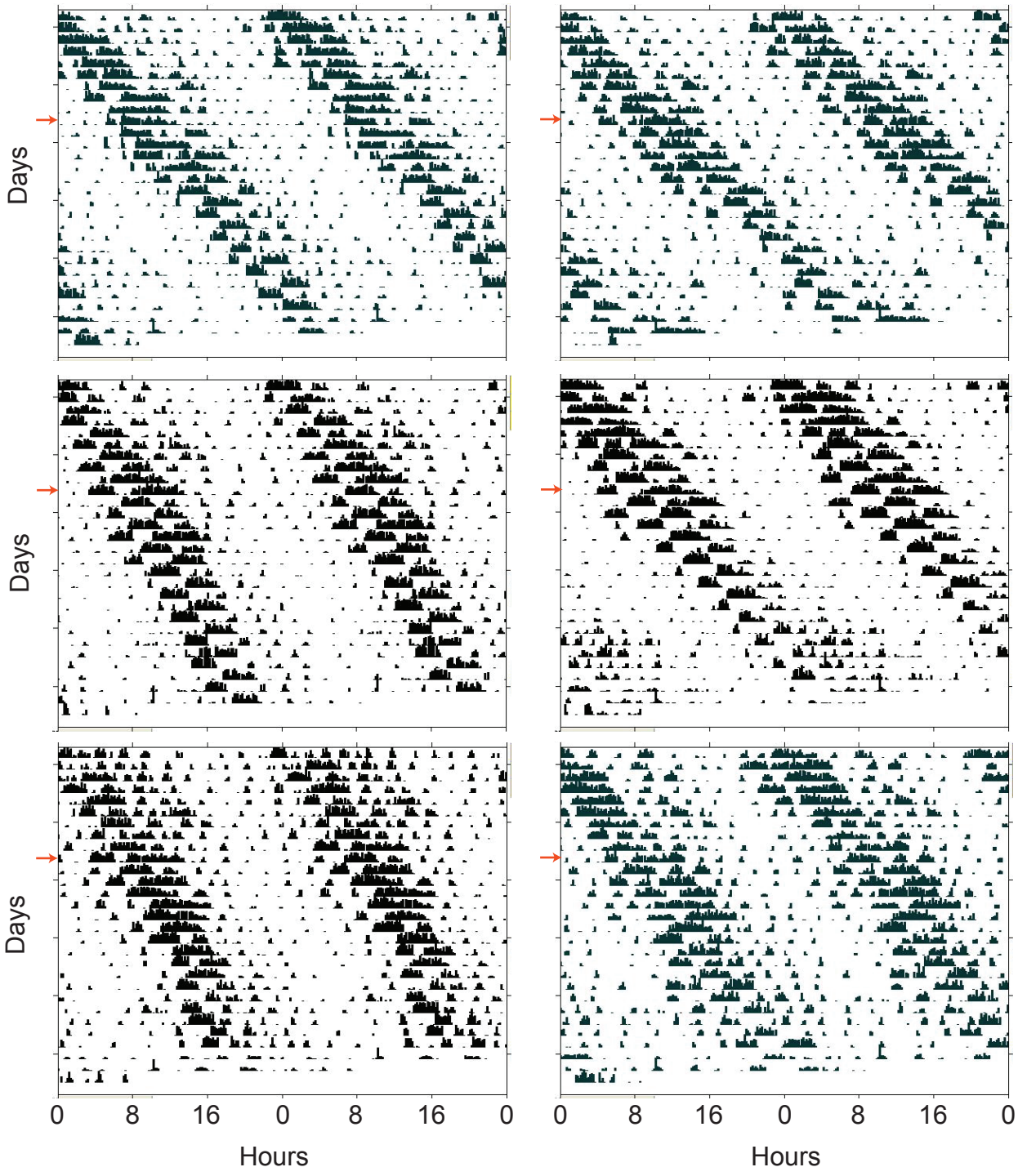
Supplementary Figure 8: Subchronic fluoxetine treatment does not rescue learning deficits nor increased depression related behavior induced by the T7 cycle
A. Mice housed in the T24 cycle showed a significant preference for the novel object whereas mice housed in the T7 cycle did not show this preference. This was also observed in mice treated for four-days with fluoxetine (one sample t-test μ_0=50%, T24 vehicle: n=6, p=0.03; T24 fluoxetine: n=7, p=0.0057, T7 vehicle: n=8, p=0.085; T7 fluoxetine: n=8, p=0.67). **B.** Mice housed in the T7 cycle show an increased amount of time spent immobile in the FST. Amount of time spent immobile is not changed by four-day fluoxetine treatment (n=7 per group, two-way ANOVA $p_{\text{light cycle}}$= 0.008). ** indicates p<0.01. Error bar indicates SEM.

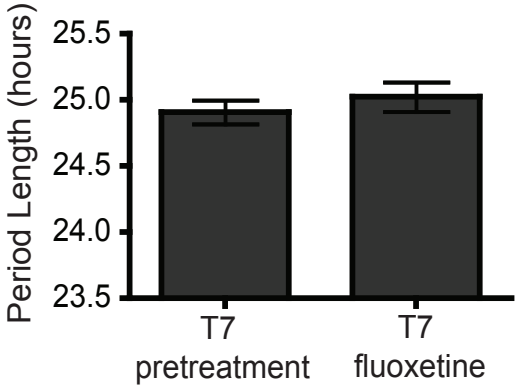


Supplementary Figure 9

Supplementary Figure 9: Administration of desipramine rescues hippocampal learning deficits

Treatment of mice housed in the T7 cycle with desipramine prior to the novel object recognition task lead to increased time spent with the novel object compared to mice treated with vehicle (one sample t-test μ_0=50%. T24 vehicle: n=12, $p<0.02$; T24 desipramine: n=12, $p<0.87$; T7 vehicle: n=12, $p<0.24$; T7 desipramine: n=12, $p<0.04$). * indicates $p< 0.05$. Error bar indicates SEM.

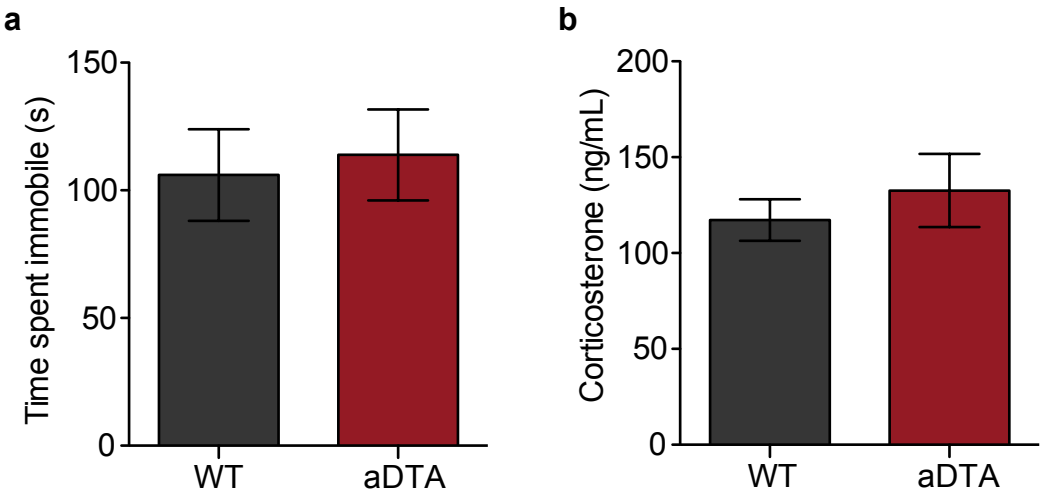




Supplementary Figure 10

Supplementary Figure 10: Fluoxetine treatment does not alter period length in the T7 light cycle

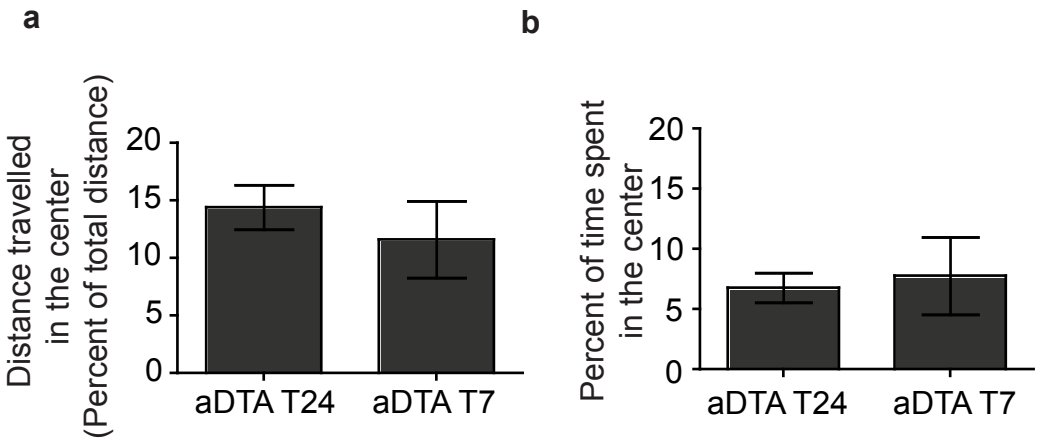
A. Actograms of general activity rhythms of mice housed under the T7 light cycle before and during fluoxetine treatment. The red arrow indicates the start of fluoxetine treatment. **B.** Period length under the T7 light cycle did not change after fluoxetine treatment (n=8 paired t-test p=0.48). Error bar indicates SEM.



Supplementary Figure 11

Supplementary Figure 11: WT and aDTA mice show no basal differences in mood related behavior

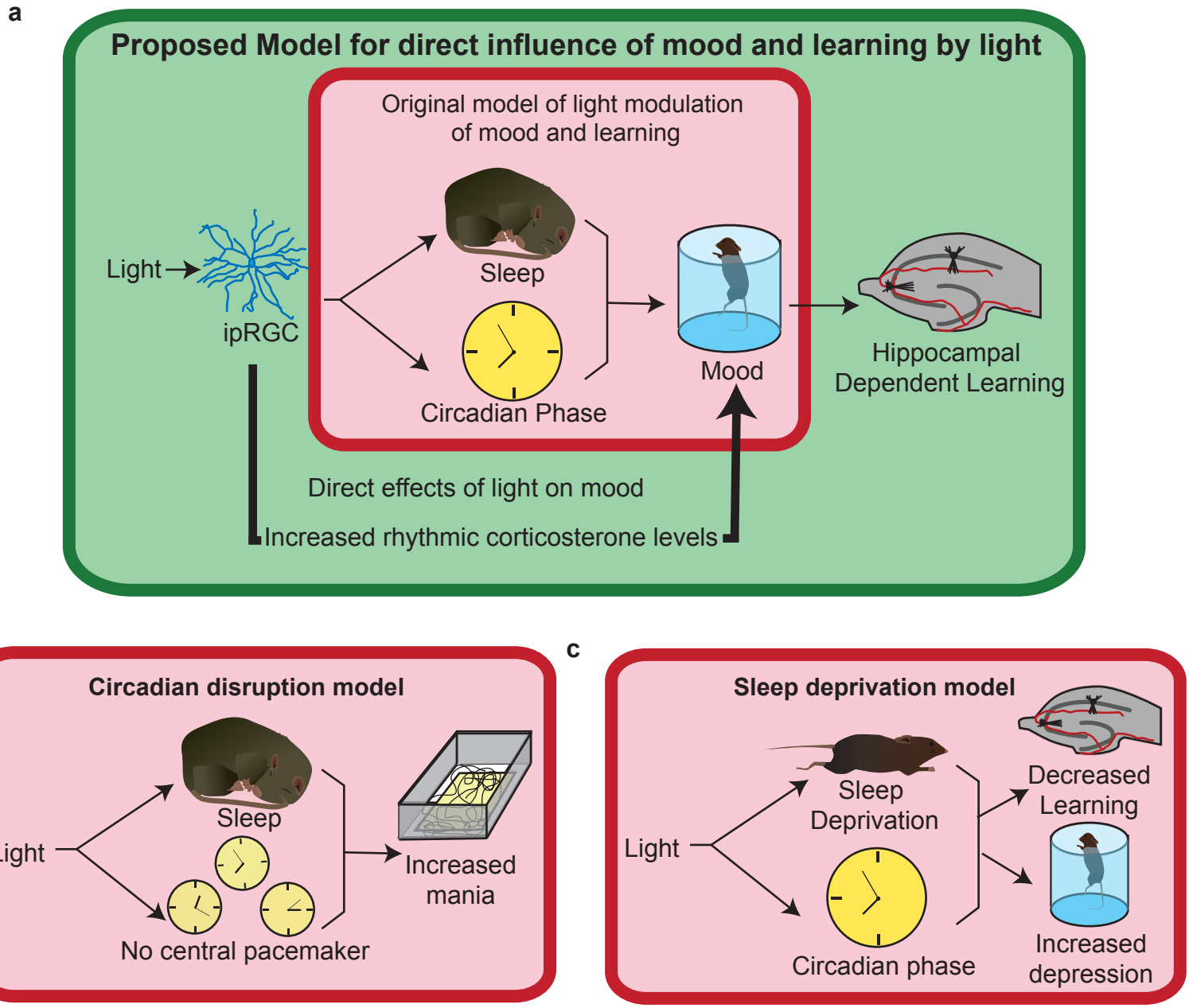
A. Time spent immobile in the FST was similar between WT and aDTA littermate mice (n=10 per group, unpaired t-test p=0.76). **B.** WT and aDTA mice showed similar basal corticosterone levels at ZT/CT13 (n=10,9 , unpaired t-test p=0.48).



Supplementary Figure 12

Supplementary Figure 12: T7 light cycle does not cause an increase in anxiety-related behaviors in aDTA mice

aDTA mice housed in the T7 cycle performed similarly to aDTA mice housed in the T24 cycle in the open field **A.** Distance travelled in the center (T24 aDTA: n=6 per group, unpaired t-test p=0.49) **B.** Percent time spent in the center (T24 aDTA: n=6 per group, unpaired t-test p=0.78). Error bar indicates SEM.



Supplementary Figure 13

Supplementary Figure 13: A model for the influence of light on mood and learning **A.** Previous studies suggested that light regulates mood and learning secondarily by first modulating sleep and circadian rhythms (red inset). In this study (green box), we reveal another pathway by which light directly affects mood through ipRGCs without disrupting sleep or causing circadian arrhythmicity. Furthermore, mood disruptions lead to learning deficits, since we were able to restore learning in mice maintained in the aberrant light environment by administering antidepressant drugs. **B.** Although, our aberrant light cycle lengthened the period of the circadian oscillator, circadian disruptions, such as those observed in the clock mutant mice show mania-like behaviors and a decrease in depression-like behavior. In contrast, our study found increased depression-like behaviors under the aberrant light conditions. This indicates that light influences depression independent of circadian rhythm disruptions. **C.** Interestingly, although sleep amounts were not affected at all in the T7 cycle, sleep deprived mice show similar deficits to mice maintained under the T7 cycle.

Supplementary References

1. Mrosovsky, N. & Hattar, S. Impaired masking responses to light in melanopsin-knockout mice. Chronobiol Int 20, 989-99 (2003).
2. Redlin, U. & Mrosovsky, N. Masking of locomotor activity in hamsters. J Comp Physiol A 184, 429-37 (1999).
3. Falls, W. A., Fox, J. H. & MacAulay, C. M. Voluntary exercise improves both learning and consolidation of cued conditioned fear in C57 mice. Behav Brain Res 207, 321-31.
4. Garcia-Capdevila, S., Portell-Cortes, I., Torras-Garcia, M., Coll-Andreu, M. & Costa-Miserachs, D. Effects of long-term voluntary exercise on learning and memory processes: dependency of the task and level of exercise. Behav Brain Res 202, 162-70 (2009).
5. Greenwood, B. N. et al. Long-term voluntary wheel running is rewarding and produces plasticity in the mesolimbic reward pathway. Behav Brain Res 217, 354-62.
6. Mistlberger, R. E. Effects of daily schedules of forced activity on free-running rhythms in the rat. J Biol Rhythms 6, 71-80 (1991).
7. Solberg, L. C., Horton, T. H. & Turek, F. W. Circadian rhythms and depression: effects of exercise in an animal model. Am J Physiol 276, R152-61 (1999).
8. Baldi, E., Lorenzini, C. A. & Corrado, B. Task solving by procedural strategies in the Morris water maze. Physiol Behav 78, 785-93 (2003).
9. Rosenthal, N. E. et al. Antidepressant effects of light in seasonal affective disorder. Am J Psychiatry 142, 163-70 (1985).
10. Virk, G., Reeves, G., Rosenthal, N. E., Sher, L. & Postolache, T. T. Short exposure to light treatment improves depression scores in patients with seasonal affective disorder: A brief report. Int J Disabil Hum Dev 8, 283-286 (2009).
11. Bouwknecht, J. A. et al. Differential effects of exposure to low-light or high-light open-field on anxiety-related behaviors: relationship to c-Fos expression in serotonergic and non-serotonergic neurons in the dorsal raphe nucleus. Brain Res Bull 72, 32-43 (2007).
12. Crawley, J. & Goodwin, F. K. Preliminary report of a simple animal behavior model for the anxiolytic effects of benzodiazepines. Pharmacol Biochem Behav 13, 167-70 (1980).
13. Gonzalez, M. M. & Aston-Jones, G. Light deprivation damages monoamine neurons and produces a depressive behavioral phenotype in rats. Proc Natl Acad Sci U S A 105, 4898-903 (2008).
14. Gong, Z. et al. Two pairs of neurons in the central brain control Drosophila innate light preference. Science 330, 499-502.