Time-of-Day-Dependent Enhancement of Adult Neurogenesis in the Hippocampus

So-ichi Tamai1, Kamon Sanada2,3, Yoshitaka Fukada1

1 Department of Biophysics and Biochemistry, Graduate School of Science, The University of Tokyo, Bunkyo-Ku, Tokyo, Japan, 2Department of Developmental Neuroscience, Osaka University Graduate School of Medicine, Suita, Osaka, Japan, 3PRESTO, Japan Science and Technology Agency, Kawaguchi, Saitama, Japan

Abstract

Background: Adult neurogenesis occurs in specific regions of the mammalian brain such as the dentate gyrus of the hippocampus. In the neurogenic region, neural progenitor cells continuously divide and give birth to new neurons. Although biological properties of neurons and glia in the hippocampus have been demonstrated to fluctuate depending on specific times of the day, it is unclear if neural progenitors and neurogenesis in the adult brain are temporally controlled within the day.

Methodology/Principal Findings: Here we demonstrate that in the dentate gyrus of the adult mouse hippocampus, the number of M-phase cells shows a day/night variation throughout the day, with a significant increase during the nighttime. The M-phase cell number is constant throughout the day in the subventricular zone of the forebrain, another site of adult neurogenesis, indicating the daily rhythm of progenitor mitosis is region-specific. Importantly, the nighttime enhancement of hippocampal progenitor mitosis is accompanied by a nighttime increase of newborn neurons.

Conclusions/Significance: These results indicate that neurogenesis in the adult hippocampus occurs in a time-of-day-dependent fashion, which may dictate daily modifications of dentate gyrus physiology.

Introduction

Neurogenesis in the mammalian brain persists through adulthood mainly within the two neurogenic structures, the dentate gyrus of the hippocampus and the subventricular zone of the forebrain [1,2]. In these areas neural progenitor cells continuously divide and give birth to new neurons [1]. Previous studies have demonstrated that behavioral and physiological stimuli such as learning [3], voluntary wheel running exercise [4], and environmental enrichment [5] enhance hippocampal neurogenesis. In addition, reproductive behaviors such as mating and pregnancy stimulate progenitor divisions and neurogenesis in the subventricular zone [6,7]. Thus, proliferation of progenitors and neurogenesis in the adult brain are dynamic processes regulated by various internal and external stimuli specific to each neurogenic region.

In the hippocampus, neuronal properties such as excitability and connectivity are known to be modulated in a time-of-day-dependent manner. For example, features of long-term potentiation show day/night fluctuations in the hippocampus [8], and hippocampus-mediated learning is facilitated in daytime [9,10]. In addition to the daily regulation of the hippocampal neurons, an apparent daily change in the number of S-phase cells has been reported in the hilus where proliferative glia reside [11]. Thus, glial proliferation seems to depend on the time of the day in the hilus. Although these observations suggest an intimate connection between the temporal information and hippocampal neurons/glia, it is unclear whether neural progenitor cells and neurogenesis in the dentate gyrus of the hippocampus are modulated in a time-of-day-dependent fashion. Given that the cell divisions in certain mammalian tissues (e.g., tongue epithelium, intestinal epithelium, and skin) associate with specific times of the day [12–14], we explored daily variations of neural progenitor divisions and neurogenesis in the adult mouse brain.

Results

Six-week-old male mice were housed individually under 12-hour light/12-hour dark cycles for 2 weeks and sacrificed at various Zeitgeber time (ZT; ZT 0 represents light on and ZT 12, light off in a 12-hour light/12-hour dark cycle). We then examined the number of dividing cells in the subgranular zone (SGZ) of the dentate gyrus by immunostaining with an antibody against phospho-histone H3 (PH3), a marker for M-phase cells. PH3-positive cells were mainly located in SGZ of the dentate gyrus where neurogenesis occurs (Figure 1A.B). The number of the PH3-positive cells in SGZ was significantly higher (p<0.0001) during the nighttime than during the daytime, showing a robust...
day/night variation of the timing of mitosis (Figure 1C). Progenitor proliferation in SGZ is known to be regulated by various stimuli such as voluntary wheel running exercise [4]. We therefore examined how the day/night variation of M-phase cells is affected by the running exercise that is mainly observed during the nighttime (Figure 2A). At ZT 6, a time when the number of M-phase cells is at the near trough in the standard housing (Figure 1), the PH3-labeled cell number was significantly elevated in SGZ of exercised animals (Figure 2B,C; \( p < 0.05 \)). By contrast, the voluntary exercise had no significant effect on the mitotic fraction at ZT 22 when the number of M-phase cells is high in the standard housing (Figure 2B,C), and exercised animals apparently showed constant levels of cell mitosis throughout the day/night cycle (Figure 2D). The time-specific effect of the voluntary exercise may be attributable to the time-restriction of the exercise or the time-of-day-dependent gating of the exercise-induced signals. Altogether, progenitor cells in SGZ show the daily change of M-phase cells that is modifiable by the nocturnal exercise.

Noticeably, day/night variations of M-phase cells is region-specific, as the number of the PH3-positive cells in the subventricular zone of the forebrain was almost constant throughout the day (Figure 3A,B). We further characterized progenitor divisions in SGZ. The number of S-phase cells remained unchanged across the day, as determined by 30-min bromodeoxyuridine (BrdU) labeling (Figure S1), which is consistent with previous reports [11,15]. These observations indicate that neural progenitor cells actively enter mitosis during the nighttime in SGZ.

The increase in M-phase cells at nighttime raises the possibility that neurons are generated in higher proportions at night. To test this hypothesis, we analyzed the number of neuronal progeny generated through the cell divisions occurring during the daytime or nighttime. Mice were injected intraperitoneally with BrdU at ZT 1 for daytime analysis or ZT 13 for nighttime in order to label the dividing progenitors and they were sacrificed 10 hours later at ZT 11 or ZT 23, respectively. Under these experimental conditions, none of the BrdU-labeled cells were immunoreactive for NeuN, a marker of mature neurons (Figure S2). This short duration of labeling (10 hours) is probably insufficient for the labeled S-phase progenitors to differentiate into mature NeuN-expressing neurons. Therefore, we examined the immunoreactivity for doublecortin (DCX), a marker of immature neurons (Figure 4A,B). Among the cells that were BrdU-labeled during the daytime (BrdU-injected at ZT 1 and harvested at ZT 11), 22% were co-labeled with DCX. On the other hand, 40% of the nighttime-labeled cells (BrdU-injected at ZT 13 and harvested at ZT 23) were DCX-positive (Figure 4C). Furthermore, when progenitors were labeled with BrdU at different times of the day (ZT 5–ZT 15 and ZT 17–ZT 27), 23% (ZT 5–ZT 15) and 56% (ZT 17–ZT 27) of BrdU-labeled cells were DCX-positive (Figure S3). These observations indicate that new neurons are generated more frequently during the nighttime (\( p < 0.05 \)), and underscore the significance of the nighttime increase in progenitor divisions.

**Discussion**

In the present study, we found that M-phase cells show a clear day/night variation, with a significant increase during the night. On the other hand, the number of S-phase progenitors remains unchanged across the day, which is consistent with previous reports [11,15]. Further, when progenitor divisions were characterized by immunostaining with an antibody against Thr161-phosphorylated form of cdc2, the immunoreactive cells showed a clear daily variation with its peak at night (Figure S4). Active cdc2 functions as an initiator of G2/M transition, and Thr161 phosphorylation is required for activation of cdc2 [16]. Therefore, we hypothesized that G2/M transition of progenitors is promoted during the night, which will result in the increase of M-phase cells. The increase in M-phase cells...
might also be attributable to an increase in length of M-phase at night, but it is less likely because mitotic delay/retardation of M-phase cells at night is not apparent, as judged from the distribution of mitotic cells in different phases (prophase, metaphase, ana/telophase) that did not change over the day (Figure S5). Based on these observations, we propose a model for the daily regulation of neural progenitor cells in the hippocampus (Figure 5). In this model, hippocampal progenitors enter the cell cycle as well as S-phase irrespective of time-of-day. The progression of these cells into M-phase is suppressed at daytime. At night, the progenitors actively enter M-phase, thereby giving rise to more neuronal progeny.

Interestingly, such a daily regulation of cell divisions is observed in certain types of tissues including tongue epithelium, intestinal epithelium, and skin [12–14], implying that the temporal regulation of neural progenitor cells in the hippocampus (Figure S5). Based on these observations, we propose a model for the daily regulation of neural progenitor cells in the hippocampus (Figure 5). In this model, hippocampal progenitors enter the cell cycle as well as S-phase irrespective of time-of-day. The progression of these cells into M-phase is suppressed at daytime. At night, the progenitors actively enter M-phase, thereby giving rise to more neuronal progeny.

Figure 2. Effects of wheel running exercise on hippocampal cell divisions. Mice were divided into two groups: one group was housed in cages with a running wheel (runner) and the other group in cages with a locked wheel (control). (A) A representative actogram of the wheel running activity from a mouse reared under the light/dark cycles. (B) Confocal images of PH3-positive cells (arrowheads) in the hippocampus at ZT 6 and ZT 22. Sections were derived from runners and controls, and were labeled with PH3 antibody (red) and with DAPI (green). Scale bar, 50 μm. (C) Total numbers of PH3-labeled cells per DG in the control and runner groups at indicated ZT (mean±s.e.m., n=6–8). *p<0.05, **p<0.01 by two-tailed student’s t-test. n.s., not significant. (D) Total numbers of PH3-labeled cells per DG in the runner group at various ZT indicated (mean±s.e.m., n=3–8 for each time point, p = 0.25 by one-way ANOVA).

doi:10.1371/journal.pone.0003835.g002

Abnormalities in circadian/daily rhythms have been suggested to underlie the development of bipolar disorder [27,28]. Notably, bipolar disorder appears to be related to dysregulated hippocampal neurogenesis. In fact, an animal model of depression with stress exhibits reduced neurogenesis in the dentate gyrus [29], while lithium, a commonly prescribed mood stabilizer for bipolar disorder, enhances hippocampal neurogenesis [30]. These studies imply that properly regulated hippocampal neurogenesis may mediate mood stabilizing effect on bipolar disorder. Importantly, daily variations of progenitor cell divisions (Figure 1C) are more apparent in the ventral dentate gyrus (Figure S6), a region contributing to the regulation of emotion [18]. These observations together provide the idea that the three events—normal daily rhythms, mood stabilization, and proper neurogenesis—are interconnected. In this context, bipolar disorder induced by aberrant daily rhythms may in part rely on abnormalities in time-of-day-dependent hippocampal neurogenesis. Understanding the mechanisms underlying the temporal regulation of neurogenesis should provide insights not only into the etiology of...
bipolar disorder, but also the physiological role of hippocampal neurogenesis.

Materials and Methods

Housing conditions and wheel running exercise

Male mice (6-week-old) were housed individually under 12-hour light/12-hour dark cycles for two weeks, in order to synchronize the phase of their internal clocks to the light/dark cycles. All the cages were placed in light-tight cabinets where temperature (23±1°C) and humidity (55±10%) were kept constant. Animals had access to food and water ad libitum. Animal experiments were conducted in accordance with guidelines set by University of Tokyo and Osaka University.

For wheel running exercise, animals were kept in standard cages for 5 days prior to the wheel running exercise. Then they were divided into two groups, one in a cage with a running wheel (runner) while the other with a locked wheel (control), and both groups were held for 9 days. Revolutions of the running wheel were registered on a computer, and the data were plotted as an actogram. In the actogram (Figure 2A), the horizontal line represents one day, and the height of black vertical bars plotted side-by-side represent the relative number of wheel revolutions within a period of 5 minutes.

Figure 3. Temporal profile in the number of M-phase cells in the subventricular zone over the day. (A) Confocal images of PH3-positive cells in the subventricular zone at ZT 6 (left panel) and ZT 22 (right panel). Sections were labeled with the anti-PH3 antibody (red) and with DAPI (green). Scale bar, 50 μm. (B) Total numbers of PH3-positive cells per subventricular zone at various ZT indicated were counted and presented as mean±s.e.m. (n=4 for each time point).

Figure 4. Nighttime stimulation of neurogenesis. (A) Confocal image of the subgranular zone of the DG labeled with antibodies against DCX (green) and BrdU (red). Scale bar, 50 μm. (B) Confocal images showing a DCX-BrdU double-labeled cell from orthogonal perspectives. (C) The percentage of DCX-BrdU double-labeled cells in the hippocampus of the mice that were injected with BrdU at ZT 1 (harvested at ZT 11) or at ZT 13 (harvested at ZT 23). Data are shown as mean±s.e.m. (n=3 for each group). *p<0.05 by two-tailed student’s t-test.
actively enter M-phase, thereby giving rise to more neuronal progeny. The progression into M-phase is suppressed in the daytime, possibly by G2 arrest. At nighttime, the progenitors enter M-phase irrespective of time-of-day. The progression into M-phase is suppressed in the daytime, possibly by G2 arrest. At nighttime, the progenitors enter M-phase irrespective of time-of-day. The progression into M-phase is suppressed in the daytime, possibly by G2 arrest. At nighttime, the progenitors enter M-phase irrespective of time-of-day. The progression into M-phase is suppressed in the daytime, possibly by G2 arrest. At nighttime, the progenitors enter M-phase irrespective of time-of-day. The progression into M-phase is suppressed in the daytime, possibly by G2 arrest. At nighttime, the progenitors enter M-phase irrespective of time-of-day. The progression into M-phase is suppressed in the daytime, possibly by G2 arrest. At nighttime, the progenitors enter M-phase irrespective of time-of-day. The progression into M-phase is suppressed in the daytime, possibly by G2 arrest. At nighttime, the progenitors enter M-phase irrespective of time-of-day. The progression into M-phase is suppressed in the daytime, possibly by G2 arrest. At nighttime, the progenitors enter M-phase irrespective of time-of-day. The progression into M-phase is suppressed in the daytime, possibly by G2 arrest. At nighttime, the progenitors enter M-phase irrespective of time-of-day. The progression into M-phase is suppressed in the daytime, possibly by G2 arrest. At nighttime, the progenitors enter M-phase irrespective of time-of-day. The progression into M-phase is suppressed in the daytime, possibly by G2 arrest. At nighttime, the progenitors enter M-phase irrespective of time-of-day. The progression into M-phase is suppressed in the daytime, possibly by G2 arrest. At nighttime, the progenitors enter M-phase irrespective of time-of-day. The progression into M-phase is suppressed in the daytime, possibly by G2 arrest. At nighttime, the progenitors enter M-phase irrespective of time-of-day. The progression into M-phase is suppressed in the daytime, possibly by G2 arrest. At nighttime, the progenitors enter M-phase irrespective of time-of-day. The progression into M-phase is suppressed in the daytime, possibly by G2 arrest. At nighttime, the progenitors enter M-phase irrespective of time-of-day. The progression into M-phase is suppressed in the daytime, possibly by G2 arrest. At nighttime, the progenitors enter M-phase irrespective of time-of-day. The progression into M-phase is suppressed in the daytime, possibly by G2 arrest. At nighttime, the progenitors enter M-phase irrespective of time-of-day. The progression into M-phase is suppressed in the daytime, possibly by G2 arrest. At nighttime, the progenitors enter M-phase irrespective of time-of-day. The progression into M-phase is suppressed in the daytime, possibly by G2 arrest. At nighttime, the progenitors enter M-phase irrespective of time-of-day. The progression into M-phase is suppressed in the daytime, possibly by G2 arrest. At nighttime, the progenitors enter M-phase irrespective of time-of-day. The progression into M-phase is suppressed in the daytime, possibly by G2 arrest. At nighttime, the progenitors enter M-phase irrespective of time-of-day. The progression into M-phase is suppressed in the daytime, possibly by G2 arrest. At nighttime, the progenitors enter M-phase irrespective of time-of-day. The progression into M-phase is suppressed in the daytime, possibly by G2 arrest. At nighttime, the progenitors enter M-phase irrespective of time-of-day. The progression into M-phase is suppressed in the daytime, possibly by G2 arrest. At nighttime, the progenitors enter M-phase irrespective of time-of-day. The progression into M-phase is suppressed in the daytime, possibly by G2 arrest. At nighttime, the progenitors enter M-phase irrespective of time-of-day. The progression into M-phase is suppressed in the daytime, possibly by G2 arrest. At nighttime, the progenitors enter M-phase irrespective of time-of-day. The progression into M-phase is suppressed in the daytime, possibly by G2 arrest. At nighttime, the progenitors enter M-phase irrespective of time-of-day. The progression into M-phase is suppressed in the daytime, possibly by G2 arrest. At nighttime, the progenitors enter M-phase irrespective of time-of-day. The progression into M-phase is suppressed in the daytime, possibly by G2 arrest. At nighttime, the progenitors enter M-phase irrespective of time-of-day. The progression into M-phase is suppressed in the daytime, possibly by G2 arrest. At nighttime, the progenitors enter M-phase irrespective of time-of-day. The progression into M-phase is suppressed in the daytime, possibly by G2 arrest. At nighttime, the progenitors enter M-phase irrespective of time-of-day. The progression into M-phase is suppressed in the daytime, possibly by G2 arrest. At nighttime, the progenitors enter M-phase irrespective of time-of-day. The progression into M-phase is suppressed in the daytime, possibly by G2 arrest. At nighttime, the progenitors enter M-phase irrespective of time-of-day. The progression into M-phase is suppressed in the daytime, possibly by G2 arrest. At nighttime, the progenitors enter M-phase irrespective of time-of-day. The progression into M-phase is suppressed in the daytime, possibly by G2 arrest. At nighttime, the progenitors enter M-phase irrespective of time-of-day. The progression into M-phase is suppressed in the daytime, possibly by G2 arrest. At nighttime, the progenitors enter M-phase irrespective of time-of-day. The progression into M-phase is suppressed in the daytime, possibly by G2 arrest. At nighttime, the progenitors enter M-phase irrespective of time-of-day. The progression into M-phase is suppressed in the daytime, possibly by G2 arrest. At nighttime, the progenitors enter M-phase irrespective of time-of-day. The progression into M-phase is suppressed in the daytime, possibly by G2 arrest. At nighttime, the progenitors enter M-phase irrespective of time-of-day. The progression into M-phase is suppressed in the daytime, possibly by G2 arrest. At nighttime, the progenitors enter M-phase irrespective of time-of-day. The progression into M-phase is suppressed in the daytime, possibly by G2 arrest. At nighttime, the progenitors enter M-phase irrespective of time-of-day. The progression into M-phase is suppressed in the daytime, possibly by G2 arrest. At nighttime, the progenitors enter M-phase irrespective of time-of-day. The progression into M-phase is suppressed in the daytime, possibly by G2 arrest. At nighttime, the progenitors enter M-phase irrespective of time-of-day. The progression into M-phase is suppressed in the daytime, possibly by G2 arrest. At nighttime, the progenitors enter M-phase irrespective of time-of-day. The progression into M-phase is suppressed in the daytime, possibly by G2 arrest. At nighttime, the progenitors enter M-phase irrespective of time-of-day. The progression into M-phase is suppressed in the daytime, possibly by G2 arrest. At nighttime, the progenitors enter M-phase irrespective of time-of-day. The progression into M-phase is suppressed in the daytime, possibly by G2 arrest. At nighttime, the progenitors enter M-phase irrespective of time-of-day. The progression into M-phase is suppressed in the daytime, possibly by G2 arrest. At nighttime, the progenitors enter M-phase irrespective of time-of-day. The progression into M-phase is suppressed in the daytime, possibly by G2 arrest. At nighttime, the progenitors enter M-phase irrespective of time-of-day. The progression into M-phase is suppressed in the daytime, possibly by G2 arrest. At nighttime, the progenitors enter M-phase irrespective of time-of-day. The progression into M-phase is suppressed in the daytime, possibly by G2 arrest. At nighttime, the progenitors enter M-phase irrespective of time-of-day. The progression into M-phase is suppressed in the daytime, possibly by G2 arrest. At nighttime, the progenitors enter M-phase irrespective of time-of-day. The progression into M-phase is suppressed in the daytime, possibly by G2 arrest. At nighttime, the progenitors enter M-phase irrespective of time-of-day. The progression into M-phase is suppressed in the daytime, possibly by G2 arrest. At nighttime, the progenitors enter M-phase irrespective of time-of-day. The progression into M-phase is suppressed in the daytime, possibly by G2 arrest. At nighttime, the progenitors enter M-phase irrespective of time-of-day. The progression into M-phase is suppressed in the daytime, possibly by G2 arrest. At nighttime, the progenitors enter M-phase irrespective of time-of-day. The progression into M-phase is suppressed in the daytime, possibly by G2 arrest. At nighttime, the progenitors enter M-phase irrespective of time-of-day. The progression into M-phase is suppressed in the daytime, possibly by G2 arrest. At nighttime, the progenitors enter M-phase irrespective of time-of-day. The progression into M-phase is suppressed in the daytime, possibly by G2 arrest. At nighttime, the progenitors enter M-phase irrespective of time-of-day. The progression into M-phase is suppressed in the daytime, possibly by G2 arrest. At nighttime, the progenitors enter M-phase irrespective of time-of-day. The progression into M-phase is suppressed in the daytime, possibly by G2 arrest. At nighttime, the progenitors enter M-phase irrespective of time-of-day. The progression into M-phase is suppressed in the daytime, possibly by G2 arrest. At nighttime, the progenitors enter M-phase irrespective of time-of-day. The progression into M-phase is suppressed in the daytime, possibly by G2 arrest. At nighttime, the progenitors enter M-phase irrespective of time-of-day. The progression into M-phase is suppressed in the daytime, possibly by G2 arrest. At nighttime, the progenitors enter M-phase irrespective of time-of-day. The progression into M-phase is suppressed in the daytime, possibly by G2 arrest. At nighttime, the progenitors enter M-phase irrespective of time-of-day. The progression into M-phase is suppressed in the daytime, possibly by G2 arrest. At nighttime, the progenitors enter M-phase irrespective of time-of-day. The progression into M-phase is suppressed in the daytime, possibly by G2 arrest. At nighttime, the progenitors enter M-phase irrespective of time-of-day. The progression into M-phase is suppressed in the daytime, possibly by G2 arrest. At nighttime, the progenitors enter M-phase irrespective of time-of-day. The progression into M-phase is suppressed in the daytime, possibly by G2 arrest. At nighttime, the progenitors enter M-phase irrespective of time-of-day. The progression into M-phase is suppressed in the daytime, possibly by G2 arrest. At nighttime, the progenitors enter M-phase irrespective of time-of-day. The progression into M-phase is suppressed in the daytime, possibly by G2 arrest. At nighttime, the progenitors enter M-phase irrespective of time-of-day. The progression into M-phase is suppressed in the daytime, possibly by G2 arrest. At nighttime, the progenitors enter M-phase irrespective of time-of-day. The progression into M-phase is suppressed in the daytime, possibly by G2 arrest. At nighttime, the progenitors enter M-phase irrespective of time-of-day. The progression into M-phase is suppressed in the daytime, possibly by G2 arrest. At nighttime, the progenitors enter M-phase irrespective of time-of-day. The progression into M-phase is suppressed in the daytime, possibly by G2 arrest. At nighttime, the progenitors enter M-phase irrespective of time-of-day. The progression into M-phase is suppressed in the daytime, possibly by G2 arrest. At nighttime, the progenitors enter M-phase whichever.
Figure S5  Mitotic stages of PH3-positive cells. (A) Representative confocal images of the PH3-positive cells in ana/telophase (top), metaphase (middle), and prophase (bottom). Scale bar, 5 µm. (B) Distribution of PH3-positive mitotic cells in different phases at various ZT indicated (mean ± s.e.m, n = 4 for each time point).

Figure S6  Daily variation of hippocampal cell proliferation. (A) Total numbers of PH3-positive cells per dorsal DG at various ZT indicated are presented as mean ± s.e.m. (n = 4 for each time point). (B) Total numbers of PH3-positive cells per ventral DG.

References
1. Ming GL, Song H (2005) Adult neurogenesis in the mammalian central nervous system. Annu Rev Neurosci 28: 223–250.
2. Alvarez-Buylla A, Garcia-Verdugo JM (2002) Neurogenesis in adult subventricular zone. J Neurosci 22: 629–634.
3. Gould E, Beylin A, Tanapat P, Reeves A, Shors TJ (1999) Learning enhances adult neurogenesis in the hippocampal formation. Nat Neurosci 2: 260–265.
4. van Praag H, Kempermann G, Gage FH (1999) Running increases cell proliferation and neurogenesis in the adult mouse dentate gyrus. Nat Neurosci 2: 266–270.
5. Kempermann G, Kuhn HG, Gage FH (1997) More hippocampal neurons in adult mice living in an enriched environment. Nature 386: 493–495.
6. Shingo T, Gegg C, Enwere E, Fujikawa H, Hassam R, et al. (2003) Pregnancy-stimulated neurogenesis in the adult female forebrain mediated by prolactin. Science 299: 117–120.
7. Mak GK, Enwere EK, Gegg C, Pakarainen T, Poutanen M, et al. (2007) Male pheromone-stimulated neurogenesis in the adult female brain: possible role in mating behavior. Nat Neurosci 10: 1003–1011.
8. Chaudhury DR, Wang LM, Colwell CS (2005) Circadian regulation of hippocampal long-term potentiation. J Biol Rhythms 20: 225–236.
9. Chaudhury DR, Colwell CS (2002) Circadian modulation of learning and memory in fear-conditioned mice. Behav Brain Res 133: 95–108.
10. Valentinuzzi VS, Menna-Barreto L, Xavier GF (2006) Effect of circadian phase on performance of rats in the Morris water maze task. J Biol Rhythms 19: 312–324.
11. Kochman LJ, Weber ET, Fornal CA, Jacobs BL (2006) Circadian variation in mouse hippocampal cell proliferation. Neurosci Lett 406: 256–259.
12. Scheving LE, Burns ER, Pauly JE, Tsai TH (1978) Circadian variation in cell division of the mouse alimentary tract, bone marrow and corneal epithelium. Anat Rec 191: 479–486.
13. Bjarnason GA, Jordan R (2002) Rhythms in human gastrointestinal mucosa and skin. Chronobiol Int 19: 129–140.
14. Bjarnason GA, Jordan RC, Sothern RB (1999) Circadian variation in the expression of cell-cycle proteins in human oral epithelium. Am J Pathol 154: 613–622.
15. Holmes MM, Galea LA, Mischberger RF, Kempermann G (2004) Adult hippocampal neurogenesis and voluntary running activity: circadian and dosedependent effects. J Neurosci Res 76: 216–222.
16. Morgan DO (1995) Principles of CDK regulation. Nature 374: 131–134.

Total numbers of PH3-positive cells per DG (the same figure as Figure 1C).

Acknowledgments
We thank Dr. M.D. Nguyen for critical reading of the manuscript.

Author Contributions
Conceived and designed the experiments: SiT KS YF. Performed the experiments: SiT. Analyzed the data: SiT. Contributed reagents/materials/analysis tools: SiT. Wrote the paper: SiT KS YF.

PLoS ONE | www.plosone.org