

Adult Hippocampal Neurogenesis and Voluntary Running Activity: Circadian and Dose-Dependent Effects

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Running activity increases cell proliferation and neurogenesis in the dentate gyrus of adult mice. The present experiment was designed to investigate whether the effect of activity on adult neurogenesis is dependent on the time of day (circadian phase) and the amount of activity. Mice received restricted access to a running wheel (0, 1, or 3 hr) at one of three times of day: the middle of the light phase (i.e., when mice are normally inactive), dark onset (i.e., when mice begin their nocturnal activity), and the middle of the dark period (i.e., when mice are in the middle of their active period). Cell proliferation and net neurogenesis were assessed after incorporation of the thymidine analog bromodeoxyuridine (BrdU) and immunohistochemical detection of BrdU and neuronal markers. Running activity significantly increased cell proliferation, cell survival, and total number of new neurons only in animals with 3 hr of wheel access during the middle of the dark period. Although activity was positively correlated with increased neurogenesis at all time points, the effects were not statistically significant in animals with wheel access at the beginning of the dark period or during the middle of the light period. These data suggest that the influence of exercise on cell proliferation and neurogenesis is modulated by both circadian phase and the amount of daily exercise, thus providing new insight into the complex relationship between physiological and behavioral factors that can mediate adult neuroplasticity.

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Physical activity and exercise have beneficial effects on brain health and cognition. Most notably, a prospective clinical study revealed that physical activity was associated with a reduced risk of cognitive impairment and dementia with age (Laurin et al., 2001). In animal studies, one of the most surprising changes linked to physical exercise is the induction of adult hippocampal neurogenesis that has been

robustly demonstrated for mice (Van Praag et al., 1999a,b; Ehninger and Kempermann, 2003). This effect is highly specific and cannot be found in the second neurogenic region of the adult brain, the olfactory system (Brown et al., 2003). In the normal neocortex of adult mice, no neurogenesis can be found; however, voluntary physical activity has an effect on the production of new macro- and microglia in the cortex (Ehninger and Kempermann, 2003). Adult hippocampal neurogenesis has been linked to aspects of hippocampal function (Gould et al., 1999; Kempermann, 2002). We have previously shown that the genetically determined baseline level of adult hippocampal neurogenesis correlates with parameters describing the acquisition phase of a hippocampus-dependent learning task (Kempermann and Gage, 2002). One theory is that the new neurons optimize the strength of the mossy fiber tract connecting the dentate gyrus with the CA3 region. Presumably, this connection has to be as narrow as possible, but as wide as necessary to cope most efficiently with the degree of complexity and novelty frequently encountered by an animal (Kempermann, 2002). Other theories have attributed storage functions (Gould et al., 1999) or a role in the process of forgetting to the new neurons (Feng et al., 2001). At present, factual knowledge on the exact function of the new neurons is scant. However, functional connections appear to be made, in that the new neurons show the electrophysiological characteristics of mature granule cells (van Praag et al., 2002).

The finding that physical activity robustly induces adult hippocampal neurogenesis appears somewhat coun-

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terintuitive in this context. If the new neurons have a specific function and if specific learning stimuli have a survival-promoting effect on the newly generated cells, why does a stimulus as broad as physical activity induce neurogenesis? Our hypothesis is that adult hippocampal neurogenesis is regulated on several levels of neuronal development, proliferation of the progenitor cells and survival of their progeny being just two of them. Thus, it is conceivable that physical activity might influence adult neurogenesis in a comparatively nonspecific way by activating progenitor cell proliferation, whereas specific learning stimuli would not affect cell proliferation but, more specifically, recruit new neurons into functional circuits. Exercise might be beneficial to hippocampal function because it increases the potential for neurogenesis.

We therefore set out to further characterize voluntary physical activity as a nonspecific modulator of adult hippocampal neurogenesis. Mice received access to a running wheel for 0, 1, or 3 hr at one of three times of day: in the middle of the light period (during which mice are normally resting), at the beginning of the dark period (the phase when mice usually become active), and in the middle of the dark period (the active period for mice). We were interested in two key variables: dose of activity (duration or intensity) and its timing (circadian phase). Dose-dependent effects on neurogenesis have been suggested for other stimuli, e.g., corticosterone (Cameron and Gould, 1994). Time-of-day effects are equally likely, given the ubiquity of circadian clock regulation in biological systems. A circadian effect on cell proliferation in particular has been reported for lobsters (Goergen et al., 2002).

Rodents with unlimited access to a running wheel run almost exclusively during their active phase at night (Mistlberger and Holmes, 2000). At the physiological level, multiple neuroendocrine systems relevant to adult neurogenesis are under circadian or behavioral state control, including the hypothalamic-pituitary-adrenal axis (Meaney et al., 1992) and growth hormone (Sinha et al., 1977), which is directly related to serum levels of insulin-like growth factor 1 (IGF-1). Given that IGF-1 might be a key molecule in regulating exercise-induced neurogenesis (Trejo et al., 2001), it is conceivable that neurogenic effects of activity could be influenced by the circadian timing of activity.

MATERIALS AND METHODS

Animals and Experimental Design

One hundred male C57Bl/6j mice (age 10 weeks) were obtained from Charles River (Quebec, Canada). Mice were housed in a 12:12-hr light:dark cycle (lights off at 1300 hr). By convention, lights on and off are designated *zeitgeber times* (ZT) 0 and 12, where *zeitgeber* (time-giver) is the light-dark cycle to which the animals are entrained, and there are 24 *zeitgeber* hours/day. Mice were group housed prior to the commencement of the experiment, at which time they were housed individually in polypropylene cages with wire mesh floors and a small plastic tube. Each cage was equipped with

a running wheel connected to a microswitch monitored continuously by computer. Wheel revolutions were summed and saved to disk at 10-min intervals. Running wheels could be locked or unlocked by the experimenter to control wheel access.

Animals were divided into three groups that received bromodeoxyuridine (BrdU) injections at ZT 6 (0700 hr), ZT 12 (1300 hr), or ZT 18 (1900 hr). Each group was further subdivided into three groups, receiving either 0, 1, or 3 hr of daily wheel access (commencing at their assigned ZT). Animals were placed into their individual cage and given 24 hr to habituate. On days 1–7, all animals received BrdU injections (see below), and then their wheels were unlocked for either 0, 1, or 3 hr. On day 8, to assess cell proliferation, half of the mice from each group were overdosed with sodium pentobarbital (Somnitol; MTC Pharmaceuticals) and perfused transcardially with cold 4% paraformaldehyde in 0.1 M phosphate-buffered saline (PBS). Numbers of animals per cell proliferation group were as follows: ZT6, 0 hr N = 6, 1 hr N = 6, 3 hr N = 6; ZT12, 0 hr N = 5, 1 hr N = 5, 3 hr N = 6; ZT18, 0 hr N = 7, 1 hr N = 6, 3 hr N = 6. The remaining animals continued in their assigned wheel access conditions for 21 more days and were perfused on day 29 to assess cell survival. As with BrdU injections, all perfusions occurred at the assigned ZT of a given experimental group (i.e. ZT6, -12, or -18). Therefore, animals did not receive wheel access on the day they were perfused. Numbers of animals per cell survival group were as follows: ZT6, 0 hr N = 6, 1 hr N = 6, 3 hr N = 6; ZT12, 0 hr N = 4, 1 hr N = 4, 3 hr N = 5; ZT18, 0 hr N = 6, 1 hr N = 5, 3 hr N = 5. Animals received their assigned wheel access for the entire duration of the experiment. All procedures were carried out in accordance with guidelines set by the Canadian Council of Animal Care and the Simon Fraser University Animal Care Committee.

BrdU Injections

BrdU (Sigma, St. Louis, MO) was dissolved in fresh 0.9% NaCl. Each mouse received single doses of 50 $\mu\text{g/g}$ body weight at a concentration of 10 mg/ml, one intraperitoneal injection per day for 7 consecutive days.

Immunohistochemistry

Immunohistochemistry for BrdU and immunofluorescent triple labeling for BrdU, NeuN, and S100 β were performed as previously described (Kuhn et al., 1997). All staining was carried out on free-floating 40- μm sections. The antibodies used were rat anti-BrdU (1:500; Harlan Seralab, Indianapolis, IN); mouse anti-NeuN (1:100; Chemicon, Temecula, CA); and rabbit anti-S100 β (1:2,500; Swant, Belinzona, Switzerland). The fluorescent secondary antibodies used were anti-rat rhodamine X, anti-mouse fluorescein isothiocyanate (FITC), and anti-rabbit CY5 (1:250; Jackson ImmunoResearch, West Grove, PA). The peroxidase method was used to determine the number of BrdU-labeled cells (ABC system, with biotinylated donkey anti-rat antibodies and diaminobenzidine as chromogen; Vector Laboratories, Burlingame, CA).

Quantification

BrdU-positive cells were counted in a one-in-six series of sections (40 μm thickness) through a $\times 40$ objective (Leica,

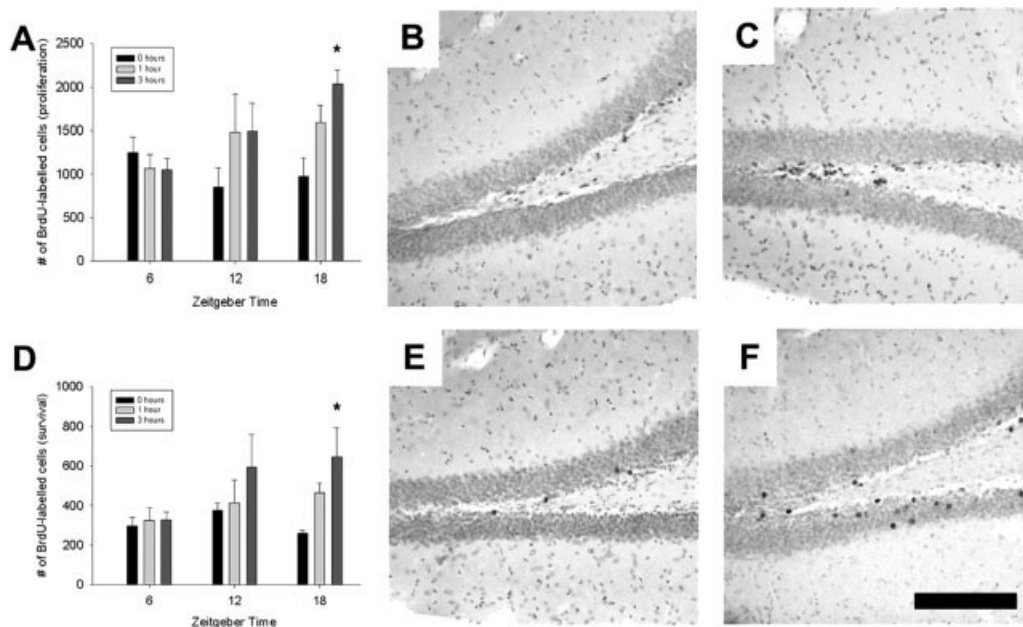


Fig. 1. Number of BrdU-positive cells in one side of the dentate gyrus. **A,D**: Total number of BrdU-positive cells 1 day (**A**) and 3 weeks (**D**) following the last BrdU injection. ZT6, mice receiving BrdU injections and wheel access at the middle of the light period (inactive phase); ZT12, mice receiving BrdU injections and wheel access at the onset of the dark period (active period); ZT18, mice receiving BrdU injections and wheel access at the middle of the dark period. 0 hours, mice that received no access to a running wheel; 1 hour, mice that received 1 hr

of daily wheel access; 3 hours, mice that received 3 hr of daily wheel access. *Significantly different ($P \leq .01$) from 0 hr wheel access control group. **B,C**: Photomicrographs of BrdU-positive cells 1 day following the last BrdU injection with 0 hr wheel access (**B**) and 3 hr wheel access (**C**) at ZT18. **E,F**: Photomicrographs of BrdU-positive cells 3 weeks following the last BrdU injection with 0 hr wheel access (**E**) and 3 hr wheel access (**F**) at ZT18. Scale bar = 150 μm .

Bensheim) throughout the rostrocaudal extent of the granule cell layer. The procedure used is a modified version of the optical fractionator principle and has been described previously (Kempermann et al., 1997). The granule cell layer was analyzed in one hemisphere for each animal, with the hemisphere (left vs. right) randomly chosen. The number of BrdU-labeled cells was multiplied by 6 to provide an estimate for the total number of BrdU-positive cells per dentate gyrus.

Analysis of Phenotypes

A one-in-six series of sections from animals surviving 21 days after the last BrdU injection was triply labeled (as described above) and analyzed using spectral confocal microscopy (Leica TCS SP2). Fifty BrdU-positive cells per animal were analyzed for coexpression of BrdU and NeuN (neuronal phenotype) or BrdU and S100 β (glial phenotype). Colabeling was verified throughout the z-axis of focus. Ratios of BrdU-positive cells colabeled with NeuN, with S100 β , or with neither NeuN nor S100 β were determined. Ratios of cells colabeled with BrdU and NeuN were then related back to the number of surviving cells (BrdU-labeled cells 3 weeks after the last injection) to provide an estimate of the total number of new neurons.

Statistical Analyses

Analysis of variance (ANOVA) tests were calculated for each of the dependent variables: number of proliferating cells,

number of surviving cells, total number of new neurons (BrdU + NeuN labeled), and mean daily activity (i.e., number of wheel revolutions). ZT6, -12, or -18 and wheel access (0, 1, or 3 hr) were the between-subjects variables in the ANOVA. Post-hoc tests utilized were Newman-Keul's unless otherwise noted. Planned comparisons were subjected to a Bonferroni correction. Pearson's product-moment correlations (r^2) were used to correlate amount of activity with number of BrdU-labeled cells. Statistical significance was set at $P \leq .05$.

RESULTS

Activity-Dependent Effects on Cell Proliferation in the Dentate Gyrus

The number of BrdU-labeled cells was assessed at two time points: at 1 day and 3 weeks after the last injection of BrdU. Values at 1 day were taken as an indicator of "proliferation," whereas the numbers at 3 weeks after BrdU are taken as a measure of "survival." At the 3-week time point, the phenotypic differentiation of the new cells was also analyzed.

There was a significant effect of the duration of wheel access on the number of BrdU-labeled cells at 1 day after BrdU [$F(2,43) = 3.583$, $P \leq .036$; Fig. 1A]. These data suggest that the effect of physical activity on cell proliferation in the dentate gyrus is dose dependent.

TABLE I. Phenotypes of BrdU-Positive Cells (Total Number and Percent $\pm$ SEM)[†]

Phenotype	ZT6			ZT12			ZT18		
	0 hr	1 hr	3 hr	0 hr	1 hr	3 hr	0 hr	1 hr	3 hr
Neuron									
No.	199.2 $\pm$ 35.6	245.7 $\pm$ 55.	227.3 $\pm$ 36.9	269.5 $\pm$ 29.4	284.8 $\pm$ 88.9	464 $\pm$ 138.6	183.9 $\pm$ 14.5	356.9 $\pm$ 46.5	509.1 $\pm$ 129.0*
Percent	66.0 $\pm$ 2.7	74.7 $\pm$ 3.4	68.0 $\pm$ 4.4	72.0 $\pm$ 3.7	67.0 $\pm$ 2.9	76.0 $\pm$ 4.2	71.0 $\pm$ 2.6	76.4 $\pm$ 2.8	79.3 $\pm$ 3.9
Astrocyte									
No.	39.8 $\pm$ 5.96	20 $\pm$ 4.5	38.8 $\pm$ 10.0	40.1 $\pm$ 8.3	33.3 $\pm$ 11.9	34.6 $\pm$ 17.3	31.2 $\pm$ 10.0	29.98 $\pm$ 3.9	27.5 $\pm$ 13.1
Percent	13.7 $\pm$ 1.5	7.3 $\pm$ 2.1	12.3 $\pm$ 2.7	10.5 $\pm$ 1.7	11.5 $\pm$ 4.4	7.2 $\pm$ 2.7	11.3 $\pm$ 3.0	7.2 $\pm$ 1.9	5.0 $\pm$ 1.9
Other									
No.	58.0 $\pm$ 6.3	57.4 $\pm$ 12.5	61.9 $\pm$ 7.3	65.5 $\pm$ 9.8	94.4 $\pm$ 32.5	95.5 $\pm$ 29.2	43.9 $\pm$ 6.0	77.5 $\pm$ 14.5	109 $\pm$ 28.0*
Percent	20.3 $\pm$ 1.7	18.0 $\pm$ 2.1	19.7 $\pm$ 2.6	17.5 $\pm$ 2.1	21.5 $\pm$ 2.6	16.8 $\pm$ 3.1	17.7 $\pm$ 3.0	16.4 $\pm$ 2.7	15.7 $\pm$ 2.7

[†]Phenotype numbers and percentages are mean $\pm$ SEM of cells labeled with either BrdU + NeuN (neuron), BrdU + S100 β (astrocyte), or BrdU alone (other). ZT6, mice receiving BrdU injections and wheel access at the middle of the light period (inactive phase); ZT12, mice receiving BrdU injections and wheel access at the onset of the dark period (active period); ZT18, mice receiving BrdU injections and wheel access at the middle of the dark period. 0 hr, mice that received no access to a running wheel; 1 hr, mice that received 1 hr of daily wheel access; 3 hr, mice that received 3 hr of daily wheel access.

*Significantly different from 0 hr control group (for the given ZT); see Results section.

In contrast, the time of day when the animal began their wheel running (ZT) had no significant main effect on the number of BrdU-positive cells at 1 day after BrdU [$F(2,43) = 2.44$, $P \leq .10$], nor was there a significant interaction between duration and ZT [$F(2,43) = 2.03$, $P \leq .11$]. A priori, we were interested in examining differences between wheel access groups at each time point; we found that 3 hr of wheel access produced a significant increase in cell proliferation at ZT18 compared with 0 hr of wheel access at ZT18 ($P \leq .002$). This was not the case at ZT6 and ZT12, for either 1- or 3-hr groups.

Activity-Dependent Effects on Cell Survival and Differentiation

At 3 weeks after BrdU, there was a significant effect of the duration of wheel access on the number of surviving BrdU-labeled cells [$F(2,38) = 4.74$, $P \leq .015$; Fig. 1D] and a trend for a main effect of ZT [$F(2,38) = 2.98$, $P \leq .06$] but no significant interaction [$F(2,38) = 1.31$, $P \leq .28$]. This can be interpreted as a dose-dependent effect of voluntary exercise on the survival of newly generated cells in the subgranular zone of the adult dentate gyrus. Comparisons between the three groups at each ZT showed that cell survival was significantly enhanced only by 3 hr of wheel access in the ZT18 group ($P \leq .002$).

Phenotypic differentiation of surviving cells was evaluated using triple-label immunofluorescence for BrdU, astrocytic marker S100 β , and neuronal marker NeuN. Again, there was a significant effect of duration of wheel access on the total number of new neurons [cells doubly labeled with BrdU and NeuN; $F(2,38) = 4.80$, $P \leq .014$; Table I, Fig. 2] and a trend for a main effect of ZT [$F(2,38) = 2.98$, $P \leq .06$] but no significant interaction [$F(2,38) = 1.38$, $P \leq .26$]. This increase was limited to the 3-hr group at ZT18 ($P \leq .003$; Table I).

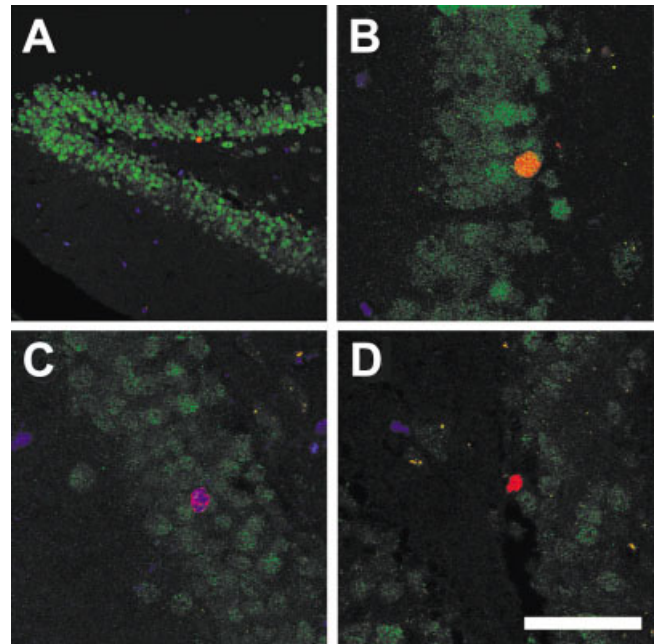


Fig. 2. Confocal images of BrdU-positive cells. Photomicrographs of sections immunofluorescence triple labeled for BrdU (red), NeuN (green), and S100 β (blue), with a representative dentate gyrus (A), BrdU-positive cell colabeled with NeuN (B), BrdU-positive cell colabeled with S100 β (C), and BrdU-positive cell not colabeled (D). All photomicrographs were taken from tissue from a ZT6 mouse. Scale bar = 150 μ m for A, 50 μ m for B–D.

Circadian Effects on Voluntary Wheel-Running Activity

Given that the duration of wheel running per se had an effect on the number of BrdU-labeled cells, we assessed group differences (between ZT groups) in the amount of running activity. We found a significant interaction between ZT and duration of wheel access [$F(4,91) = 12.28$, $P \leq .001$] and main effects for wheel access duration

[$F(2,91) = 122.00$, $P \leq .001$] and ZT [$F(2,91) = 24.84$, $P \leq .001$]. Post-hoc tests revealed that ZT6 3-hr mice ran significantly less than both ZT12 3-hr ($P \leq .001$) and ZT18 3-hr ($P \leq .001$), but there were no significant differences between any of the 1-hr groups (Fig. 3B). Importantly, ZT12 and ZT18 mice did not statistically differ in the amount of daily wheel running-activity at any level of wheel access.

Correlations Among Wheel Running, Time of Day, and Adult Neurogenesis

Mean daily activity was positively correlated with cell proliferation only in ZT18 mice ($r = 0.57$, $P \leq .01$) but not in ZT6 ($r = -0.05$, $P \leq .84$) or ZT12 ($r = 0.21$, $P \leq .43$) mice. However, the total number of new neurons was significantly correlated with mean daily activity regardless of ZT (ZT6, $r = 0.61$, $P \leq .01$; ZT12, $r = 0.65$, $P \leq .02$; ZT18, $r = 0.81$, $P \leq .005$). Thus running activity was associated with increased cell proliferation only at ZT18, but with increased net neurogenesis at all times tested (with the strongest correlation at ZT18).

DISCUSSION

The results of the present experiment extend earlier reports that voluntary running activity significantly increases cell proliferation, cell survival, and total number of new neurons born in the dentate gyrus of adult mice (Van Praag et al., 1999a,b). The present data demonstrate that this effect is both time and dose dependent. Animals permitted 3 hr of wheel access during the middle of their circadian active phase (ZT18) had significantly increased levels of neurogenic activity. Animals permitted to run at other circadian time points either did not have increased neurogenic activity (during the light/inactive phase; ZT6) or these effects failed to reach statistical significance (onset of the active phase; ZT12). It is interesting that mean daily running activity was positively correlated with cell survival at all time points, demonstrating that these activity-induced effects are also dose dependent. That we obtained significant correlations between running and cell survival at all times tested but not with cell proliferation (only at ZT18) suggests that running may enhance cell survival independently of its well known effects on cell proliferation. Taken together, these data suggest that, although activity may be associated with increased neurogenesis at all times of day, this increase was statistically significant only at one time point tested (the middle of the active phase; ZT18). Thus time of day, or more specifically circadian phase, can influence activity-induced increases in cell proliferation and neurogenesis.

Circadian Rhythms and Scheduled Running Activity

In the present experiment, mice were permitted a set duration of wheel access at a particular circadian phase. Mice are nocturnal animals that normally exhibit intense wheel-running activity during the dark period and little or

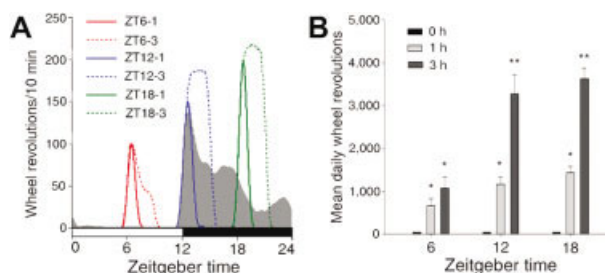


Fig. 3. Wheel-running activity expressed during controlled wheel access. **A:** Average waveform of wheel revolutions/10 min across a 24-hr period in mice with controlled wheel access. Shading represents an average of 40 mice (not used in the present experiment) with ad libitum wheel access. **B:** Mean daily activity (number of wheel revolutions) broken down by amount of wheel access and ZT. ZT12 and ZT18 mice with 3 hr of wheel access ran significantly more than all other groups. ZT6, mice receiving BrdU injections and wheel access at the middle of the light period (inactive phase); ZT12, mice receiving BrdU injections and wheel access at the onset of the dark period (active period); ZT18, mice receiving BrdU injections and wheel access at the middle of the dark period. 0h, mice that received no access to a running wheel; 1h, mice that received 1 hr of daily wheel access; 3h, mice that received 3 hr of daily wheel access. *Significantly different from animals with 0 hr wheel access ($P \leq .05$). **Significantly different from animals with 0 or 1 hr of wheel access ($P \leq .05$).

no wheel running during the light phase. The present data demonstrate that mice will voluntarily run in a wheel regardless of whether wheel access is at the onset of the dark period (ZT12) or at the middle of the dark period (ZT18). We were particularly interested in whether animals that had wheel access *only* during the light (i.e., inactive) phase (ZT6) would run at levels comparable to those of their dark period counterparts (ZT12 and ZT18). Not surprisingly, ZT6 mice do run at higher rates for the first hour of wheel access but run less during the second or third hour of wheel access (Fig. 3A). Therefore, it is possible that we did not see an increase in cell proliferation and neurogenesis in ZT6 mice because activity fell below a “critical level.” However, ZT12 animals had the same levels of activity as ZT18 animals. Although in both ZT12 and ZT18 an increase in cell proliferation and neurogenesis was found (Fig. 1), the effects were statistically significant *only* in the ZT18 group. Arguably, these data do not make a strong case for a circadian effect independent of the dose of activity. Rather these data suggest that neurogenic activity is not merely dependent on duration or amount of activity but is also under the control of circadian phase.

However, there is a second level to this consideration. Given that all activity within the present experiment was voluntary, it could be argued that the amount of activity expressed at a given circadian time is irrelevant as mice had equivalent access to a running wheel. The running wheel was used only to measure voluntary activity. With this interpretation, circadian regulation of activity-induced neurogenesis is striking (ZT6 compared with ZT18). This suggests that, under natural conditions, there may be a circadian rhythm of neurogenesis that is medi-

ated by activity. Thus mice voluntarily run less at particular circadian phases and, as such, may experience little or no activity-induced neurogenic activity. Specific correlations within ZT groups show that activity is positively correlated with cell proliferation only at ZT18. Although activity is positively correlated with cell survival at all time points tested (ZT6, -12, and -18), these correlations are greatest (though not significantly) at ZT18, further supporting a role for circadian rhythms in the regulation of activity-induced neurogenesis.

Circadian Regulation of Putative Physiological Factors

The dependence of activity-induced neurogenesis on the circadian time of wheel running may provide additional insight into neurochemical factors regulating neurogenesis in the adult organism. Of particular interest are cases in which activity has differential effects on neurogenesis and neurochemical variables. For example, serotonin has been implicated as one neurotransmitter that promotes neurogenesis (Brezun and Daszuta, 1999, 2000). Intriguingly, extracellular serotonin levels are enhanced in at least some brain regions when wheel running is stimulated during the usual inactive phase (corresponding to ZT6 in nocturnal rodents) but are significantly suppressed when running is stimulated during the latter half of the usual active phase (corresponding to ZT18 in our experiment; Dudley et al., 1998).

It is well established that plasma corticosterone levels rise late in the rest phase of the circadian rest–activity cycle and peak early in the active phase, in both nocturnal and diurnal animals (Ottewiller et al., 1979; Weber et al., 2000; Barriga et al., 2001). Furthermore, corticosterone release appears to be enhanced in association with running at any circadian time (Sellers et al., 1988). Baseline levels of serum corticosterone, however, do not correlate with adult hippocampal neurogenesis (Kempermann and Gage, 2002), and mice living in an enriched environment had significantly increased corticosterone levels and increased adult hippocampal neurogenesis (Kempermann et al., 2002). Thus, moderate levels of corticosterone might be associated with both increased cell survival and number of new neurons. Taken with data demonstrating that high levels of corticosterone are associated with decreased levels of cell proliferation (Cameron and Gould, 1994; Tanapat et al., 2001; Holmes and Galea, 2002), this is consistent with a possible curvilinear relationship between corticosterone and neurogenesis. However, corticosterone is unlikely to be the only factor mediating activity-dependent effects on adult hippocampal neurogenesis. Circulating IGF-1 has been suggested as a key mediator of this regulation (Aberg et al., 2000; Carro et al., 2000; Trejo et al., 2001), but many other types of circulating factors might be involved, such as fibroblast growth factor 2 (Tao et al., 1996; Gomez-Pinilla et al., 1997), prolactin (Shingo et al., 2003), or possibly erythropoietin (Shingo et al., 2001).

The present data demonstrate that the effects of exercise on cell proliferation and neurogenesis are dependent on circadian phase and on the amount of daily

exercise. This complex interaction appears to be an important aspect of how “activity” might regulate adult hippocampal neurogenesis. This characterization might turn out to be helpful if one attempts to further elucidate why and how something as general as activity is beneficial. Our data give some new insight into the regulation of neurogenesis, indicating a complex relationship between physiological and behavioral factors that can mediate neurogenesis. They suggest the existence of modulatory interdependencies, indicating that the induction of adult hippocampal neurogenesis by voluntary wheel running might be a more specific event than was initially thought. However, what these specific aspects are remains to be elucidated.

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REFERENCES

- Aberg MA, Aberg ND, Hedbacker H, Oscarsson J, Eriksson PS. 2000. Peripheral infusion of IGF-I selectively induces neurogenesis in the adult rat hippocampus. *J Neurosci* 20:2896–2903.
- Barriga C, Martin MI, Tabla R, Ortega E, Rodriguez AB. 2001. Circadian rhythm of melatonin, corticosterone and phagocytosis: effect of stress. *J Pineal Res* 30:180–187.
- Brezun JM, Daszuta A. 1999. Depletion in serotonin decreases neurogenesis in the dentate gyrus and the subventricular zone of adult rats. *Neuroscience* 89:999–1002.
- Brezun JM, Daszuta A. 2000. Serotonin may stimulate granule cell proliferation in the adult hippocampus, as observed in rats grafted with foetal raphe neurons. *Eur J Neurosci* 12:391–396.
- Brown J, Cooper-Kuhn CM, Kempermann G, van Praag H, Winkler J, Gage FH, Kuhn HG. 2003. Enriched environment and physical activity stimulate hippocampal but not olfactory bulb neurogenesis. *Eur J Neurosci* 23:2042–2046.
- Cameron HA, Gould E. 1994. Adult neurogenesis is regulated by adrenal steroids in the dentate gyrus. *Neuroscience* 61:203–209.
- Carro E, Nunez A, Busiguina S, Torres-Aleman I. 2000. Circulating insulin-like growth factor I mediates effects of exercise on the brain. *J Neurosci* 20:2926–2933.
- Dudley TE, DiNardo LA, Glass JD. 1998. Endogenous regulation of serotonin release in the hamster suprachiasmatic nucleus. *J Neurosci* 18:5045–5052.
- Ehninger D, Kempermann G. 2003. Regional effects of wheel running and environmental enrichment on cell genesis and microglial proliferation in the adult murine neocortex. *Cereb Cortex* 13:845–851.
- Feng R, Rampon C, Tang YP, Shrom D, Jin J, Kyin M, Sopher B, Martin GM, Kim SH, Langdon RB, Sisodia SS, Tsien JZ. 2001. Deficient neurogenesis in forebrain-specific presenilin-1 knockout mice is associated with reduced clearance of hippocampal memory traces. *Neuron* 32:911–926.
- Goergen EM, Bagay LA, Rehm K, Benton JL, Beltz BS. 2002. Circadian control of neurogenesis. *J Neurobiol* 53:90–95.

- Gomez-Pinilla F, Dao L, So V. 1997. Physical exercise induces FGF-2 and its mRNA in the hippocampus. *Brain Res* 764:1–8.
- Gould E, Tanapat P, Hastings NB, Shors TJ. 1999. Neurogenesis in adulthood: a possible role in learning. *Trends Cogn Sci* 3:186–192.
- Holmes MM, Galea LA. 2002. Defensive behavior and hippocampal cell proliferation: differential modulation by naltrexone during stress. *Behav Neurosci* 116:160–168.
- Kempermann G. 2002. Why new neurons? Possible functions for adult hippocampal neurogenesis. *J Neurosci* 22:635–638.
- Kempermann G, Gage FH. 2002. Genetic determinants of adult hippocampal neurogenesis correlate with acquisition, but not probe trial performance in the water maze task. *Eur J Neurosci* 16:129–136.
- Kempermann G, Kuhn HG, Gage FH. 1997. Genetic influence on neurogenesis in the dentate gyrus of adult mice. *Proc Natl Acad Sci USA* 94:10409–10414.
- Kempermann G, Gast D, Gage FH. 2002. Neuroplasticity in old age: sustained fivefold induction of hippocampal neurogenesis by long-term environmental enrichment. *Ann Neurol* 52:135–143.
- Kuhn HG, Winkler J, Kempermann G, Thal LJ, Gage FH. 1997. Epidermal growth factor and fibroblast growth factor-2 have different effects on neural progenitors in the adult rat brain. *J Neurosci* 17:5820–5829.
- Laurin D, Verreault R, Lindsay J, MacPherson K, Rockwood K. 2001. Physical activity and risk of cognitive impairment and dementia in elderly persons. *Arch Neurol* 58:498–504.
- Meaney MJ, Aitken DH, Sharma S, Viau V. 1992. Basal ACTH, corticosterone and corticosterone-binding globulin levels over the diurnal cycle, and age-related changes in hippocampal type I and type II corticosteroid receptor binding capacity in young and aged, handled and nonhandled rats. *Neuroendocrinology* 55:204–213.
- Mistlberger RE, Holmes MM. 2000. Behavioral feedback regulation of circadian rhythm phase angle in light-dark entrained mice. *Am J Physiol Regul Integr Comp Physiol* 279:R813–R821.
- Ottewill JE, Meier AH, Russo AC, Frenze ME. 1979. Circadian rhythms of plasma corticosterone binding activity in the rat and the mouse. *Acta Endocrinol* 91:150–157.
- Sellers TL, Jaussi AW, Yang HT, Heninger RW, Winder WW. 1988. Effect of the exercise-induced increase in glucocorticoids on endurance in the rat. *J Appl Physiol* 65:173–178.
- Shingo T, Sorokan ST, Shimazaki T, Weiss S. 2001. Erythropoietin regulates the in vitro and in vivo production of neuronal progenitors by mammalian forebrain neural stem cells. *J Neurosci* 21:9733–9743.
- Shingo T, Gregg C, Enwere E, Fujikawa H, Hassam R, Geary C, Cross JC, Weiss S. 2003. Pregnancy-stimulated neurogenesis in the adult female forebrain mediated by prolactin. *Science* 299:117–120.
- Sinha YN, Salocks CB, Wickes MA, Vanderlaan WP. 1977. Serum and pituitary concentrations of prolactin and growth hormone in mice during a twenty-four hour period. *Endocrinology* 100:786–791.
- Tanapat P, Hastings NB, Rydel TA, Galea LA, Gould E. 2001. Exposure to fox odor inhibits cell proliferation in the hippocampus of adult rats via an adrenal hormone-dependent mechanism. *J Comp Neurol* 437:496–504.
- Tao Y, Black IB, DiCicco-Bloom E. 1996. Neurogenesis in neonatal rat brain is regulated by peripheral injection of basic fibroblast growth factor (bFGF). *J Comp Neurol* 376:653–663.
- Trejo JL, Carro E, Torres-Aleman I. 2001. Circulating insulin-like growth factor I mediates exercise-induced increases in the number of new neurons in the adult hippocampus. *J Neurosci* 21:1628–1634.
- Van Praag H, Kempermann G, Gage FH. 1999a. Running increases cell proliferation and neurogenesis in the adult mouse dentate gyrus. *Nat Neurosci* 2:266–270.
- Van Praag H, Christie BR, Sejnowski TJ, Gage FH. 1999b. Running enhances neurogenesis, learning and long-term potentiation in mice. *Proc Natl Acad Sci USA* 96:13427–13431.
- Van Praag H, Schinder AF, Christie BR, Toni N, Palmer TD, Gage FH. 2002. Functional neurogenesis in the adult hippocampus. *Nature* 415:1030–1034.
- Weber B, Lewicka S, Deuschle M, Colla M, Vecsei P, Heuser I. 2000. Increased diurnal plasma concentrations of cortisone in depressed patients. *J Clin Endocrinol Metab* 85:1133–1136.