

## Host Circadian Clock as a Control Point in Tumor Progression

Elisabeth Filipksi, Verdun M. King, XiaoMei Li, Teresa G. Granda, Marie-Christine Mormont, XuHui Liu, Bruno Claustrat, Michael H. Hastings, Francis Lévi

**Background:** The circadian timing system controlled by the suprachiasmatic nuclei (SCN) of the hypothalamus regulates daily rhythms of motor activity and adrenocortical secretion. An alteration in these rhythms is associated with poor survival of patients with metastatic colorectal or breast cancer. We developed a mouse model to investigate the consequences of severe circadian dysfunction upon tumor growth. **Methods:** The SCN of mice were destroyed by bilateral electrolytic lesions, and body activity and body temperature were recorded with a radio transmitter implanted into the peritoneal cavity. Plasma corticosterone levels and circulating lymphocyte counts were measured (n = 75 with SCN lesions, n = 64 sham-operated). Complete SCN destruction was ascertained postmortem. Mice were inoculated with implants of Glasgow osteosarcoma (n = 16 with SCN lesions, n = 12 sham-operated) or pancreatic adenocarcinoma (n = 13 with SCN lesions, n = 13 sham-operated) tumors to determine the effects of altered circadian rhythms on tumor progression. Time series for body temperature and rest-activity patterns were analyzed by spectral analysis and cosinor analysis. Parametric data were compared by the use of analysis of variance (ANOVA) and survival curves with the log-rank test. All statistical tests were two-sided. **Results:** The 24-hour rest-activity cycle was ablated and the daily rhythms of serum corticosterone level and lymphocyte count were markedly altered in 75 mice with complete SCN destruction as compared with 64 sham-operated mice (two-way ANOVA for corticosterone: sampling time effect  $P < .001$ , lesion effect  $P = .001$ , and time  $\times$  lesion interaction  $P < .001$ ; for lymphocytes  $P = .001$ ,  $.002$ , and  $.002$  respectively). Body tempera-

ture rhythm was suppressed in 60 of the 75 mice with SCN lesions ( $P < .001$ ). Both types of tumors grew two to three times faster in mice with SCN lesions than in sham-operated mice (two-way ANOVA:  $P < .001$  for lesion and for tumor effects;  $P = .21$  for lesion  $\times$  tumor effect interaction). Survival of mice with SCN lesions was statistically significantly shorter compared with that of sham-operated mice (log-rank  $P = .0062$ ). **Conclusions:** Disruption of circadian rhythms in mice was associated with accelerated growth of malignant tumors of two types, suggesting that the host circadian clock may play an important role in endogenous control of tumor progression. [J Natl Cancer Inst 2002;94:690-7]

The delivery of anticancer agents at specific circadian stages was shown to concurrently improve tolerability and/or antitumor efficacy in several murine tumor models (1-5). The clinical

*Affiliations of authors:* E. Filipksi, X. Li, T. G. Granda, M.-C. Mormont, X. Liu, F. Lévi, Institut National de la Santé et de la Recherche Médicale (INSERM), Equipe Propre INSERM 0118 "Cancer chronotherapeutics" (Université Paris XI), Paul Brousse Hospital, Villejuif, France; V. M. King, Department of Anatomy, University of Cambridge, U.K.; B. Claustrat, Service Radiopharmacie et Radioanalyse, Hôpital Neurocardiologique, Lyon, France; M. H. Hastings, Laboratory of Molecular Biology, Medical Research Council, Cambridge, U.K.

*Correspondence to:* Francis Lévi, M.D., Ph.D., Institut National de la Santé et de la Recherche Médicale (INSERM) Equipe Propre INSERM 0118 "Cancer chronotherapeutics" (Université Paris XI), Paul Brousse Hospital, 94800 Villejuif, France (e-mail: chronbio@club-internet.fr).

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relevance of this principle was demonstrated in multicenter randomized trials, as the tolerability of mucosae and that of sensory nerves were improved fivefold and twofold, respectively, with chronomodulated chemotherapy as compared with drug infusion at a constant rate. Moreover, the antitumor activity of the chronotherapy regimen also showed enhancement that was statistically significant ( $P < .003$ ) (6,7). Nevertheless, approximately 30% of cancer patients with metastatic disease display rhythm alterations, a condition that could influence the disease outcome (8,9). Thus, we recently found that severe alterations in the rest-activity circadian rhythm predicted a fivefold increase in the risk of death in patients with metastatic colorectal cancers as compared with those with normal rest-activity patterns (10). Similarly, an abnormal cortisol rhythm predicted a doubling of the risk of death in patients with metastatic breast cancer as compared with those with a normal cortisol pattern (11). Most importantly, the predictive value of alterations in these rhythms predicting poor survival outcome was independent from all known clinical factors in both studies.

Mammalian circadian rhythms are generated by interconnected molecular loops involving specific genes controlling the circadian clock [reviewed in (12)]. A central pacemaker consisting of suprachiasmatic nuclei (SCN), located in the hypothalamus, coordinates these rhythms and directly controls the rest-activity circadian cycle (13). The ablation of SCN results in the suppression of these rhythms in several rodent species, whereas transplantation of SCN restores these rhythms in animals with prior SCN lesions (14). The SCN are responsible for the adjustment of the whole circadian time structure to the photoperiodic environment, the main synchronizer of bodily rhythms (14). As a result, most biochemical or molecular cell processes display predictable changes along the 24-hour time scale, which can modulate anticancer drug pharmacology (15).

We hypothesized that severe circadian dysfunction could accelerate tumor progression *per se* and tested this possibility in mice with ablation of SCN before tumor inoculation.

## METHODS

### Study Design

In a first series of experiments, we studied the effect of SCN destruction on the rhythms in rest-activity body temperature, plasma corticosterone concentration, and circulating lymphocyte count in 188 B6D2F<sub>1</sub> mice. Mice were randomly allocated to receive sham operation (64 animals) or SCN lesions (124 animals). Fourteen mice from the latter group were eliminated from the study as a consequence of postoperative death or obesity resulting from the destruction of other hypothalamic nuclei. In a second study, B6D2F<sub>1</sub> mice were randomly assigned to receive either sham operation (25 animals) or introduction of SCN lesions [45 animals before receiving a fragment of either Glasgow osteosarcoma (GOS) (16) or the more slowly growing pancreatic adenocarcinoma (P03) (17)]. Two mice with SCN lesions were monitored as non-tumor-bearing controls, and 14 mice were eliminated from the study because of postoperative death or obesity. Both tumor cell lines are known to display a circadian rhythm-modulated susceptibility to chemotherapy (18,19). Male B6D2F<sub>1</sub> mice, 5–6 weeks old, were purchased from Charles River (L'Arbresle, France). All the experiments were performed in mice synchronized to 12 hours of light and 12 hours of darkness (LD 12:12, with lights on from 6:00 AM to 6:00 PM) to

allow for an SCN-independent photoperiodic synchronization, which may be the case in clinically relevant situations. All procedures were performed in accordance with French guidelines for experimental animal care.

### Monitoring Rhythms

Locomotor activity and body temperature were measured every 10 minutes throughout each experiment with the use of a radio transmitter (Physio Tel, TA 10 TA-F20; Data Sciences, St. Paul, MN) implanted into the peritoneal cavity of each mouse.

### SCN Destruction and Fourier Analysis

The SCN were destroyed by bilateral electrolytic lesion (20) (1 mA for 4 seconds; stereotaxic coordinates: approximately [anterior/posterior] on the Bregma line, mediolateral [ $\pm$  0.2 mm of midline], dorsoventral [0.55 mm below dural surface], incisor bar at the same level as the ear bars). Sham-operated animals underwent the same stereotaxic procedure without electrolytic lesions. Effective SCN lesions were identified by the loss of any dominant periodicity ( $\tau$ ) of locomotor activity in the circadian domain (20 h  $<$   $\tau$   $<$  28 h) with Fourier transform spectral analysis following visual inspection (21). Complete SCN destruction was ascertained postmortem in all mice for pathologic changes by investigators who had no knowledge of any data on these mice with respect to rest-activity cycles, temperature patterns, or blood variables. Histologic methods involved both Nissl stain of the hypothalamus tissue and immunostaining for peptide histidine-isoleucine (PHI) (20,22,23).

### Anesthesia

Mice were anesthetized with a single intraperitoneal injection of a 0.5-mL solution of 10 g of 2,2,2-tribromoethanol (Fluka, Saint-Quentin-Fallavier, France) in 10 mL of 2-methyl-2-butanol (Fluka) diluted 1:39 in 0.9% NaCl.

### Assessment of Corticosterone Levels and Lymphocyte Counts

Blood samples were taken from separate groups of either animals with SCN lesions or sham-operated animals at one of six different times, 4 hours apart, over a 24-hour span (3, 7, 11, 15, 19, or 23 hours after light onset [HALO]), 3–7 weeks after introducing SCN lesions. Lymphocyte count was determined with a Cell Dye® 3500R (Abbott Diagnostics, Rungis, France), and corticosterone concentration was measured by radioimmunoassay (RIA) (5  $\mu$ L of plasma was extracted with 3 mL of diethyl ether; the residue was dissolved in 100  $\mu$ L of assay buffer before incubation with 1,2,6,7 [<sup>3</sup>H]corticosterone [Amersham, Orsay, France] and rabbit anti-corticosterone antibody [Valbiotech, Paris, France]).

### Tumor Models

GOS and P03 tumor cells were provided by the Research Center of Aventis Pharma (Vitry sur Seine, France). Both tumors were maintained in C57BL/6 female mice and passaged every 2 weeks (for GOS) or every 4 weeks (for P03) as bilateral subcutaneous implants. Three passages were required before tumor transplantation in the experimental mice. Tumor fragments of size 4  $\times$  4 mm<sup>2</sup> were prepared from dissected tumors grown

in the donor mice and kept in Hanks' medium for approximately 1 hour. They were freshly implanted subcutaneously in each flank of male B6D2F<sub>1</sub> mice with a 12-gauge trocar. The experiment followed a 2 × 2 factorial design to test the role of SCN lesions, that of tumor type, and an interaction term, with regard to tumor growth and survival.

Tumor size was measured three times a week using a caliper. Tumor weight (mg) was estimated from two perpendicular measurements (mm): tumor weight = (length × width<sup>2</sup>)/2.

Mice with tumor weight reaching approximately 2 g were sacrificed for ethical reasons and considered as dead from tumor progression on this date. Four mice (two with SCN lesions and two sham-operated) were not inoculated with tumor and served as healthy controls.

### Statistical Analysis

Means and 95% confidence intervals were computed for each set of parameters. Intergroup differences were evaluated statistically using multiple-way analyses of variance (ANOVA). The effect of SCN lesions on tumor growth was assessed with 2-way ANOVA for repeated measures and followed by Student's *t* test. Time series were analyzed by spectral analysis (Fourier transform analysis) using Mathcad 6.0. Statistical significance of circadian rhythmicity was further documented by cosinor analysis (24). This method characterized a rhythm by the parameters of the fitted cosine function best approximating all data. A period  $\tau = 24$  hours was determined *a priori*. The rhythm characteristics estimated by this linear least squares method include the mesor (*M*, rhythm-adjusted mean), the double amplitude (2*A*, difference between minimum and maximum of fitted cosine function), and the acrophase ( $\emptyset$ , time of maximum in fitted cosine function, with light onset as  $\emptyset$  reference, so that units were in HALO). A rhythm was detected if the null hypothesis was rejected with  $P < .05$ ; however, *A* and  $\emptyset$  were considered as valid imputations if  $.05 < P < .10$ , as the statistical power could possibly be affected by the number of mice studied. Survival was computed with the Kaplan–Meier method, and differences in survival were validated with the log-rank test. All statistical tests were two-sided. All standard statistical tests were performed using SPSS for Windows software (Statistical Package for Social Sciences, Chicago, IL).

## RESULTS

### Disruption of Circadian Coordination

The rest–activity cycle and the temperature rhythms of all sham-operated mice were marked and were found to be regular from one day to the next (a representative example is given in Fig. 1, A, left panel, top and bottom, respectively). Spectral analysis adjusted best-fitting sinusoidal functions with decreasing periods to the data. The initial period (fundamental *T*) corresponded to the whole length of the time series (168 h in the examples shown in Fig. 1). The subsequent harmonic periods tested were *T*/2, *T*/3, *T*/4,....*T*/50. The 7<sup>th</sup> harmonic (*T*/7) corresponded to a period of 24 h. Thus, this method identified the 24-h period as the most prominent one among the initial 50 harmonics tested with corresponding periods ranging from 168 to 3.36 hours (Fig. 1, A, right panel, top and bottom). The rest–activity cycle was suppressed in 79 of 110 mice with SCN lesions (a representative example is given in Fig. 1, B, top left panel). Spectral analysis did not reveal any 24-hour periodicity

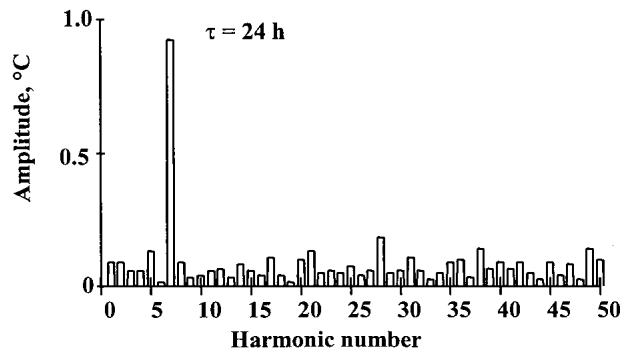
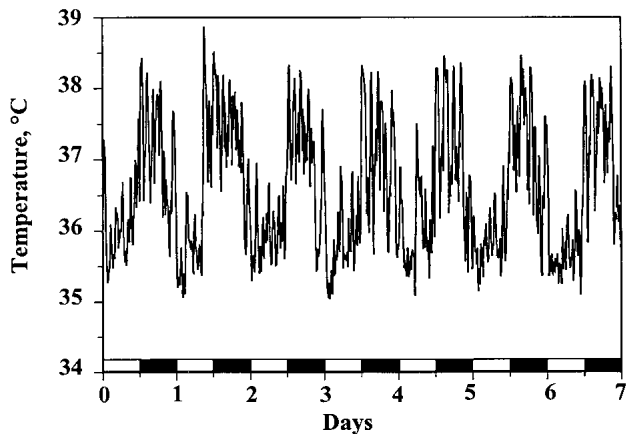
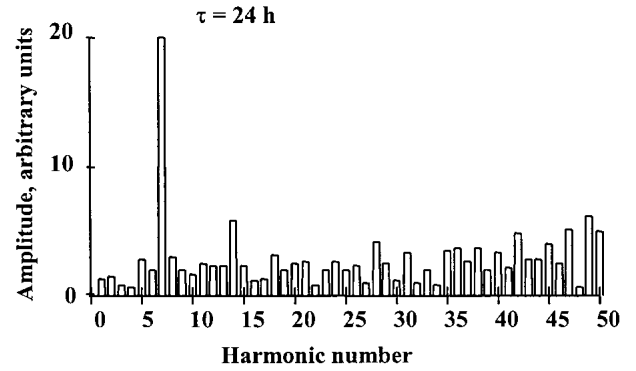
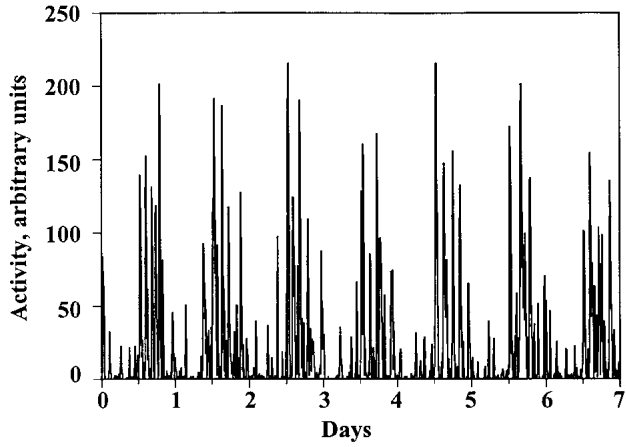
that stood out among the initial 50 harmonics (Fig. 1, B, top right panel). A postmortem histologic study ascertained complete SCN destruction in 75 animals. Only the animals with histologically verified SCN destruction were included in the SCN lesion group for comparison. None of the sham-operated mice were excluded from the analysis. Body temperature rhythm was suppressed in 60 of the 75 mice with histologically verified SCN lesions (Fig. 1, B, lower left and right panels). An atypical body temperature rhythm was found in 15 of these 75 mice. It was confirmed by a dominant 24-hour periodicity with Fourier transform analysis and a statistically significant 24-hour rhythm with cosinor analysis ( $P < .001$ ). The amplitude of temperature, however, was greatly decreased as compared with that of sham-operated mice; the maximum (acrophase) occurred between mid-light and early dark as compared with mid-dark in sham-operated animals (data not shown).

The corticosterone rhythm was similar in sham-operated mice and in control (unoperated) mice, with a peak located at the light–dark transition. The mean value at peak was roughly sevenfold greater than the mean trough value. The rhythm was altered in mice with lesions, with a mean peak value that was roughly twofold greater than the mean trough value. Furthermore, the peak was advanced to mid-light (Fig. 2, A). Two-way ANOVA validated statistically significant effects of SCN lesion ( $P = .001$ ), sampling time ( $P < .001$ ), and lesion × time interaction ( $P < .001$ ). According to cosinor analysis, 24-hour mean value was decreased from 28.5 ng/mL (in sham-operated mice) to 20.8 ng/mL (in mice with SCN lesions), and the circadian amplitude was reduced from 23.4 to 6.3 ng/mL (Table 1). The mean lymphocyte count of sham-operated mice reached maximum values in late dark to mid-light, twice as high as that of nadir in early dark. The rhythm was damped in mice with SCN lesions, with a mean value at peak (at late dark) exceeding by roughly 50% the mean value at trough (at late light) (Fig. 2, B). Two-way ANOVA demonstrated statistically significant effects of SCN lesion ( $P = .002$ ), sampling time ( $P = .001$ ), and lesion × time interaction ( $P = .002$ ).

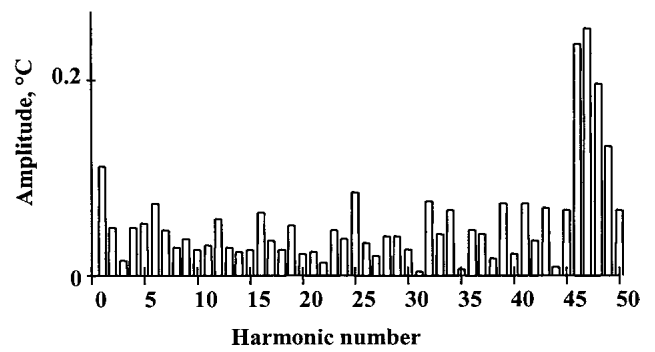
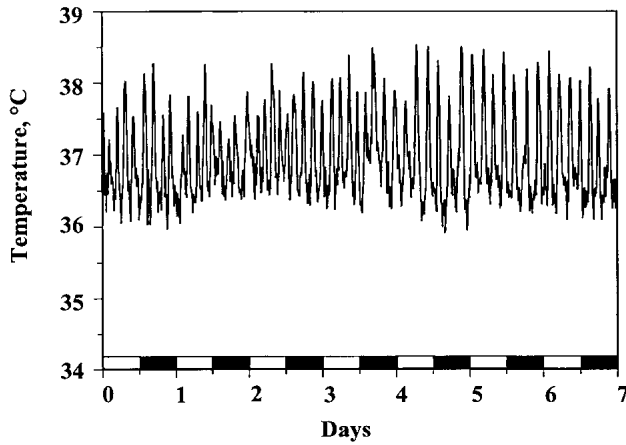
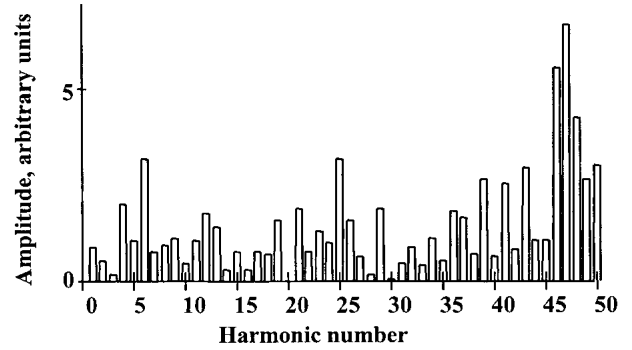
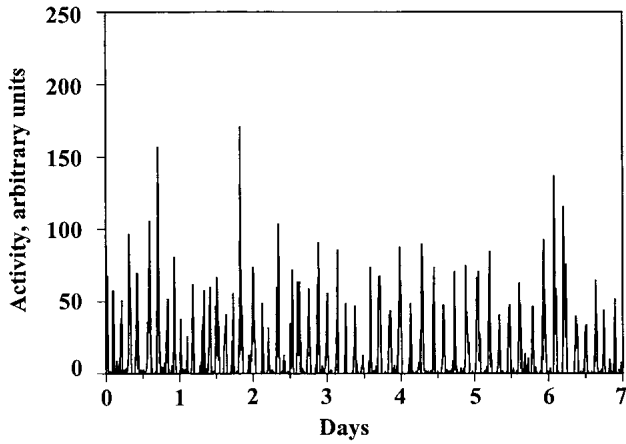
The severe damping of amplitude and the phase displacement in mice with SCN lesions were further validated with cosinor analysis (Table 1). The mesor (approximately 24-h mean value) and the amplitude (approximately half of the circadian variability above or below the mesor) were statistically significantly reduced in mice with SCN lesions for both variables. The acrophase (timing of maximum, with light onset as phase reference) of plasma corticosterone and that of lymphocyte count, respectively, occurred 90 and approximately 420 minutes earlier in animals with SCN lesions as compared with those in sham-

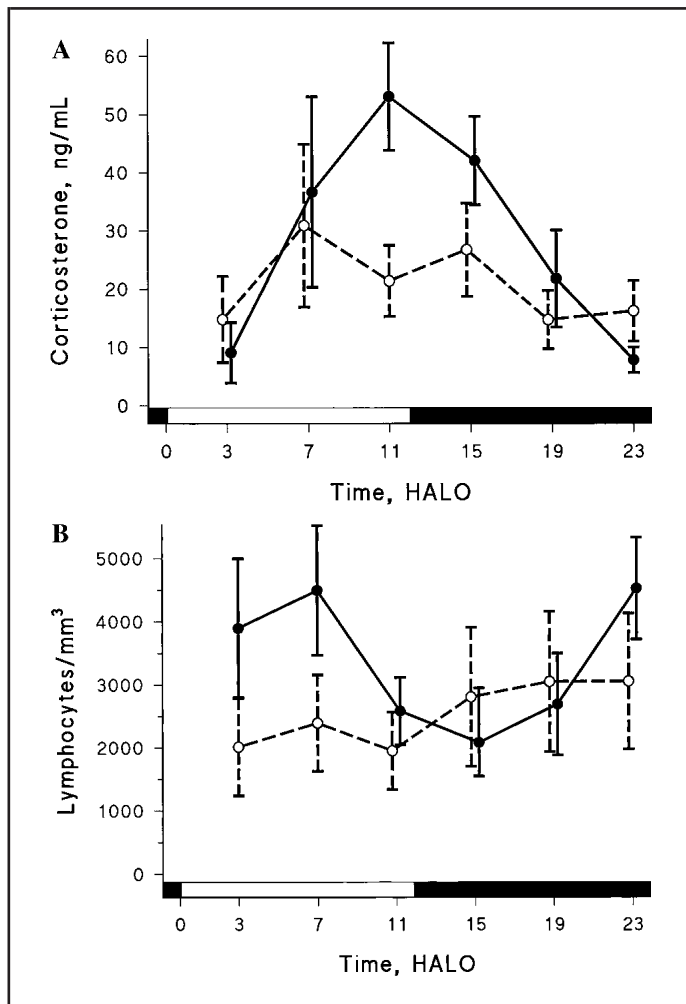
**Fig. 1.** Rest–activity and temperature rhythms in a sham-operated mouse and in a mouse with lesions in suprachiasmatic nuclei (SCN). Examples of a 7-day rest–activity (left, top) and temperature records (left, bottom) and corresponding results from Fourier transform analysis (right panels) in a sham-operated mouse (panel A) and in a mouse with SCN destruction (panel B). A rhythmic pattern was clearly recognizable in the sham-operated mouse, with Fourier analysis indicating a dominant 24-hour period. No rhythm was obvious in the mouse with SCN lesion, and no 24-hour period stood out in Fourier analysis. □ indicates the 12-hour light span and ■ indicates the 12-hour dark span on the *x*-axis. For the spectral analysis (right panels), each vertical bar indicates the amplitude (ordinate) of the harmonic corresponding to the period tested (abscissa). In these examples, the fundamental period is 168 hours (duration of time series). The 7<sup>th</sup> harmonic corresponds to a period of 24 hours (168/7), and the 50<sup>th</sup> harmonic corresponds to a period of 3.37 hours (168/50).

A



B





**Fig. 2.** Circulating corticosterone and lymphocyte rhythms in sham-operated mice and mice with lesions in suprachiasmatic nuclei (SCN). Serum concentration of corticosterone (**panel A**) and lymphocyte count (**panel B**) are shown as a function of sampling time. Each point represents the mean and 95% confidence intervals of 10–11 sham-operated mice (**solid circles**) or 12–14 mice with SCN lesions (**open circles**). Sampling time is expressed in hours after light onset (HALO). □ indicates the 12-hour light span and ■ indicates the 12-hour dark span. Two-way analysis of variance of the corticosterone data validated statistically significant effects of SCN lesion ( $P = .001$ ), sampling time ( $P < .001$ ), and lesion  $\times$  time interaction ( $P < .001$ ). Similar results were found for lymphocyte count ( $P = .002$ ,  $.001$ , and  $.002$ , respectively).

operated mice. Cosinor analysis confirmed the marked alterations that SCN lesions produced in circadian rhythms in peripheral blood.

### Relevance of Circadian Coordination for Tumor Growth

Twenty-nine mice with histologically confirmed SCN lesions had received either GOS ( $n = 16$ ) or P03 ( $n = 13$ ) tumors. Sham-operated animals had been transplanted with GOS ( $n = 12$ ) or P03 ( $n = 13$ ) tumors. The results indicated a highly statistically significant effect of SCN lesions and tumor type on tumor size (2-way ANOVA,  $P < .001$  for lesion and for tumor effects), without any statistically significant interaction (lesion  $\times$  tumor) ( $P = .21$ ). Accelerated growth of each tumor in mice with SCN lesions was further statistically validated with one-way ANOVA (GOS,  $P = .004$ ; P03,  $P < .001$ ). In GOS tumor-bearing mice, tumor growth rate was faster in mice with SCN lesions than in sham-operated mice. On day 12, i.e., before the death of the first animal, mean tumor weight was 1443 mg (95% confidence interval [CI] = 1009 mg to 1844 mg) versus 490 mg (95% CI = 273 mg to 707 mg) in mice with SCN lesions and in sham-operated mice, respectively (for  $t$  test,  $P = .002$ ) (Fig. 3, A). In mice bearing the slower growing P03 tumors, tumor growth rate was faster in mice with SCN lesions as compared with that in the sham-operated mice. On day 22, i.e., before the death of the first animal, mean tumor weights were 1447 mg (95% CI = 695 mg to 2199 mg) in mice with SCN lesions and 749 mg (95% CI = 458 mg to 1040 mg) in sham-operated mice (for  $t$  test,  $P = .05$ ) (Fig. 3, B). Similarly, the survival of sham-operated mice was statistically significantly longer than that of mice with SCN lesions (median survival time in days: sham-operated mice = 26 (95% CI = 23 to 29); mice with SCN lesions = 22 (95% CI = 19 to 25);  $P$  for log-rank test =  $.0062$ ). As we have observed in our previous studies, the mice with histologically verified partial SCN lesions ( $n = 4$ ) displayed a normal circadian pattern in rest–activity and body temperature. Tumor growth rate in these mice was similar with that in control mice (data not shown).

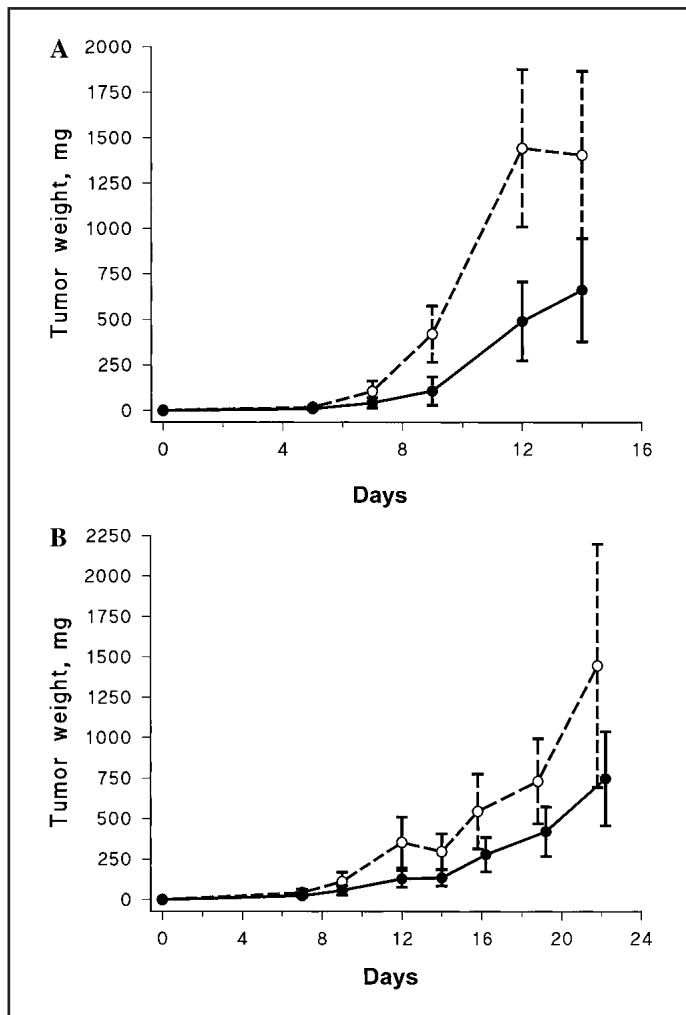
### DISCUSSION

This study demonstrates for the first time the functional role of SCN with regard to tumor growth. The SCN have long been regarded as the master circadian clock in view of their role in the rest–activity cycle (14). Nevertheless, their effect on other

**Table 1.** Results from cosinor analysis of plasma corticosterone levels and circulating lymphocyte counts in sham-operated mice ( $n = 64$ ) and in mice with lesions in suprachiasmatic nuclei (SCN) ( $n = 75$ )\*

Variable	Mice	$P$	Mesor mean (CI)	Amplitude mean (CI)	Acrophase, h:min mean (CI)
Corticosterone, ng/mL	Sham-operated	<.001	28.5 (25.2 to 31.8)	23.4 (17.5 to 29.4)	11:48 (10:50 to 12:50)
	With SCN lesions	.03	20.8 (17.5 to 24.1)	6.3 (0.6 to 12.1)	10:18 (6:00 to 14:40)
Lymphocytes, cells/mm <sup>3</sup>	Sham-operated	<.001	3394 (3066 to 3722)	1245 (651 to 1840)	2:48 (0:55 to 4:40)
	With SCN lesions	.08	2536 (2196 to 2876)	562 (242 to 882)	19:42 (14:42 to 24:42)

\* $P$  value of rhythm detection is given for each variable and group of mice. All circadian parameters (mesor, amplitude, and acrophase) are given as mean and 95% confidence intervals (CIs). The definitions of the terms are given in the “Methods” section. The mesor (estimated 24-h mean value) and the amplitude (estimated half of the circadian variability above or below the mesor) were significantly reduced in mice with SCN lesions for both variables. The acrophase (timing when the level is maximum, with light onset as a phase reference) of plasma corticosterone and that of lymphocyte count, respectively, occurred 90 and approximately 420 minutes earlier in animals with SCN lesions as compared with those in sham-operated mice. Cosinor analysis confirmed the marked alterations that SCN lesions produced in circadian rhythms in peripheral blood.



**Fig. 3.** Tumor growth in sham-operated and mice with lesions in suprachiasmatic nuclei (SCN). Mean tumor weights are shown with their respective 95% confidence intervals. Tumor growth in mice with Glasgow osteosarcoma (GOS) (**panel A**) or pancreatic adenocarcinoma (P03) (**panel B**). Sham-operated mice are represented by **solid circles** and mice with SCN lesions by **open circles**. Accelerated growth of both tumors in mice with SCN lesions was statistically validated with ANOVA (GOS,  $P = .004$ ; P03,  $P < .001$ ).

rhythms is less well established. Here, we 1) confirmed that SCN destruction ablated the rest-activity cycle in all the mice and 2) documented weak circadian coordination of several other rhythms. A persistent yet atypical body temperature rhythm was statistically validated in 20% of the mice. The amplitude was damped, and the maximum temperature occurred during the light span rather than during darkness, as was previously observed for this and other rodent species (25,26). A significant circadian variation was also found for plasma corticosterone and circulating lymphocyte count in the group of mice with SCN lesions. Both rhythms, however, displayed reduced amplitude and altered phase as compared with those in sham-operated mice. Two hypotheses could account for the occurrence of circadian rhythms despite SCN destruction. Either the light/dark cycle may be partly driving the abnormally phased rhythms in temperature, corticosterone, and lymphocyte count, although it had no overt effect on activity, or peripheral circadian oscillators may be able to function in the absence of the central clock control of the SCN, yet with poor synchrony (27). Indeed, func-

tional circadian oscillators have been demonstrated recently in cultured mammalian cells and peripheral tissues (28–30).

SCN ablation accelerated malignant growth by twofold to threefold for two established tumors with different proliferation rates. In addition, tumor growth in the mice with partial SCN destruction was similar to that in controls, illustrating the need for complete SCN destruction to accelerate tumor proliferation. This remote antitumor effect of SCN may be mediated by either endocrine or neuroanatomic communications between the SCN and peripheral targets. For example, the existence of paracrine secretions from the SCN has been demonstrated by neural grafting experiments in which encapsulated SCN tissue can restore circadian patterning to locomotor activity in hosts with lesions (31). Additionally, the SCN have widespread anatomic associations with the autonomic nervous system (32), which may influence host responses and/or factors in the host environment that regulate tumor growth. Both tumor models have displayed sensitivity to immunotherapeutic agents (16,33), which suggests that SCN destruction could favor tumor progression through immune suppression. The mean lymphopenia that we observed in mice with SCN lesions was associated with a reduced mean serum corticosterone level, a finding divergent from what was expected, because lymphopenia usually results from glucocorticoid supplementation (34). This and the differential phase-shifting and amplitude-reduction effects of SCN destruction on serum corticosterone and lymphocyte count rhythms support distinct SCN control pathways of immunologic and adrenal rhythmic functions (35,36). Recent studies (30) highlight a role for corticosteroids in coordinating peripheral circadian oscillators located in a variety of tissues. The loss of temporal organization across tissues arising from SCN lesion-induced corticosteroid dysrhythmia may contribute to enhanced tumor growth. It is necessary to investigate further whether the endocrine or immune effects triggered by SCN lesions could differently affect transplanted or endogenous tumors. Yet, chemically induced carcinomas in rats were promoted by nonspecific methods of circadian alterations, such as continuous light exposure (37).

The current findings show that release from circadian regulation causes a dramatic acceleration of malignant growth, a result in line with recent clinical reports. In a study involving 200 patients with metastatic colorectal cancer, survival at 4 years was 22% in the patients with a marked rest-activity cycle as compared with 11% in those with damped or altered rhythm. As indicated by multivariate analysis, the prognostic value of circadian rhythmicity in rest-activity was independent of well-known prognostic factors such as performance status, number of organs with metastasis, or degree of liver tissue replacement by tumor (10). The relationship between salivary cortisol rhythm and survival was explored in 104 patients with previously treated metastatic breast cancer. A normal cortisol pattern, with peak concentration at 8:00 AM and decline thereafter, was found in 37% of the patients. This group had a 4-year survival rate of 55% as compared with 26% in the patients with an altered cortisol secretion pattern. Cortisol slope was an independent predictor of survival, as determined by the multivariate analysis (11). Two large epidemiologic studies (38–41) further showed that disrupted circadian coordination induced by light at night increased the risk of developing breast cancer in women. Although the clinical data support the idea that circadian clock function partly controls several stages of tumor

development, our experimental model clearly demonstrates a specific role for the hypothalamic clock with regard to cancer proliferation.

We expect that improved understanding of the biologic dynamics of neoplasia will stem from an examination of the temporal interplay between the central SCN clock, peripheral tissue-based oscillators, and malignant processes and will lead to novel therapeutic approaches to cancer.

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## NOTES

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