Relationship between locomotor activity rhythm and corticosterone levels during HCC development, progression, and treatment in a mouse model

Soha A. Hassan1,2 | Amira A.H. Ali1 | Mona Yassine1 | Dennis Sohn3 | Martina Pfeffer1 | Reiner U. Jänicke3 | Horst-Werner Korf4 | Charlotte von Gall1

1Institute of Anatomy II, Medical Faculty, Heinrich-Heine-University, Düsseldorf, Germany
2Zoology Department, Faculty of Science, Suez University, Suez, Egypt
3Laboratory of Molecular Radiooncology, Clinic and Policlinic for Radiation Therapy and Radiooncology, Medical Faculty of Heinrich-Heine-University, Düsseldorf, Germany
4Institute of Anatomy I, Medical Faculty, Heinrich-Heine-University, Düsseldorf, Germany

Correspondence
Horst-Werner Korf, Institute of Anatomy I, Medical Faculty, Heinrich-Heine-University, Düsseldorf, Germany. Email: korf@uni-duesseldorf.de

Funding information
DAAD

Abstract
Cancer-related fatigue (CRF) and stress are common symptoms in cancer patients and represent early side effects of cancer treatment which affect the life quality of the patients. CRF may partly depend on disruption of the circadian rhythm. Locomotor activity and corticosterone rhythms are two important circadian outputs which can be used to analyze possible effects on the circadian function during cancer development and treatment. The present study analyzes the relationship between locomotor activity rhythm, corticosterone levels, hepatocellular carcinoma (HCC) development, and radiotherapy treatment in a mouse model. HCC was induced in mice by single injection of diethylnitrosamine (DEN) and chronic treatment of phenobarbital in drinking water. Another group received chronic phenobarbital treatment only. Tumor bearing animals were divided randomly into four groups irradiated at four different Zeitgeber time points. Spontaneous locomotor activity was recorded continuously; serum corticosterone levels and p-ERK immunoreaction in the suprachiasmatic nucleus (SCN) were investigated. Phenobarbital treated mice showed damped corticosterone levels and a less stable 24 hours activity rhythm as well as an increase in activity during the light phase, reminiscent of sleep disruption. The tumor mice showed an increase in corticosterone level during the inactive phase and decreased activity during the dark phase, reminiscent of CRF. After irradiation, corticosterone levels were further increased and locomotor activity rhythms were disrupted. Lowest corticosterone levels were observed after irradiation during the early light phase; thus, this time might be the best to apply radiotherapy in order to minimize side effects.

KEYWORDS
cancer-related fatigue, corticosterone, hepatocellular carcinoma, locomotor activity, mouse model, p-ERK, radiotherapy
1 | INTRODUCTION

Cancer-related fatigue (CRF) is one of the most pronounced symptoms in cancer patients and one of the early chronic side effects of cancer treatment. Many cancer patients suffer from severe sleep problems, disrupted locomotor activity rhythm and cortisol levels and a decrease in life quality which accompany CRF, particularly after radiotherapy treatment.1-5

Sleep regulation involves two intertwined processes: the homeostatic regulation and the output from the circadian system.6 Thus, CRF may partly depend on disruption of the circadian rhythm. In humans and all mammalian species, the circadian rhythm is generated by the suprachiasmatic nucleus (SCN) of the hypothalamus and synchronized to the environmental rhythms by external cues called “Zeitgebers.” The most prominent “Zeitgeber” is the external light-dark (LD) cycle, the photoperiod. Photoperiod stimuli are perceived by the retina and are transmitted to the SCN via the retino-hypothalamic tract (RHT), which utilizes glutamate and the neuropeptide pituitary adenylate cyclase-activating peptide (PACAP) as neurotransmitters. Signal transduction of these neurotransmitters activates the extracellular signal-regulated kinase 1/2 (ERK1/2) pathway which plays an important role in conveying photic information to the SCN. In addition, phosphorylated ERK (p-ERK) interacts directly with some clock proteins (e.g. BMAL1) and activates other transcription factors such as CREB which in turn modulates the expression of other clock genes (e.g. Per).7-11 Via multiple output pathways, the circadian system controls a variety of overt body functions including the sleep-wake cycle, locomotor activity, and hormone secretion (e.g. glucocorticoids).7,12,13

The locomotor activity rhythm is considered a reliable marker of the circadian output and the main index that reflects the sleep-wake cycle and the general behavior in mammals.14-16 Recording the locomotor activity patterns has been used to analyze possible dysfunctions of the circadian system in patients with cancer and after applying the cancer treatment protocols.17,18

Rhythmic secretion of glucocorticoids is another important marker for circadian output. Under chronic stress, the hypothalamus-pituitary-adrenal (HPA) axis is stimulated and induces glucocorticoid secretion19,20 which used as a stress biomarker. Thus, there is a strong relation between the circadian system and stress.21 Stress is observed in many patients suffering from cancer.3 Recently, there is growing evidence that the sleep disruption in cancer patients may be closely related to dysfunction of circadian rhythms including glucocorticoid secretion.2

The primary aim of the present study is to analyze how tumor development and irradiation treatment affect spontaneous locomotor activity rhythms. As experimental animal model, Per2::luc mice were selected based on previous studies.22 Hepatocellular carcinomas (HCCs) were induced by diethylnitrosamine (DEN) injection and phenobarbital in drinking water to accelerate tumor development. Spontaneous locomotor activity rhythms were recorded before and after therapeutic irradiation. In addition, serum corticosterone levels and p-ERK immunoreaction, a marker for rhythmic SCN neuronal activity, were analyzed. To explore the effect of chronic phenobarbital treatment without tumor induction, the analyses were also performed with mice which received phenobarbital in the drinking water only and compared with mice that received neither the DEN injection nor the chronic phenobarbital treatment.

2 | MATERIAL AND METHOD

2.1 | Experimental animals and tumor induction

The experiments were conducted using transgenic Per2::luc mice on a C57BL6/J background according to federal guidelines and Directive 2010/63/EU of the European Union of animal care. The experiments were approved by the Regierungspräsidium Darmstadt and LANUV (Reference number: AZ 81-02.04.2018-A146). The appropriate measures were taken to reduce the pain or discomfort of experimental animals. Male offspring were selected at the age of 14 days and received a single intraperitoneal injection with DEN (10 mg/kg, Sigma Aldrich) to induce HCC. To promote HCC induction, the animals were chronically treated with phenobarbital (PB) (Luminal, Desitin, Hamburg, Germany) added to the drinking water at a concentration of 0.05% according to.23,24 Tumor development was confirmed by postmortem inspection (Figure S1). These animals constituted the tumor group. A second group comprised animals which received chronic PB treatment but were not injected with DEN (phenobarbital group). A third group comprised animals which received neither DEN injection nor PB treatment (control group). HCC developed in animals of the tumor group at the age of 7-10 months. Food and drinking water (either with or without PB) were supplied ad libitum. All animals were kept under normal light-dark (LD) cycle (12:12).

2.2 | Experimental design

Recordings of the locomotor activity were started in 7-month-old animals and lasted for 30 consecutive days. Recordings were performed for 5 animals in the control and phenobarbital groups and for 20 animals in the tumor group under normal LD cycle (12:12). ZT00 (at 05.00 AM) defines the light on and ZT12 (at 05.00 PM) defines the light off. The 20 animals of the tumor group were randomly selected for treatment with irradiation at four different Zeitgeber time (ZT) points (ZT02, ZT08, ZT14 and ZT20) (5 animals/time
point). One day after irradiation, the locomotor activity was recorded for five consecutive days (Figure S2).

2.3 | Irradiation

Animals of the tumor group were irradiated at ZT02 (2 hours after light on), ZT08, ZT14 (2 hours after light off) and ZT20. Before irradiation, the animals were deeply anesthetized by intraperitoneal injection of a mixture of ketamine (100 mg/kg, Inresa, Freiburg, Germany) and xylazine (10 mg/kg, Rompun 2%, Bayer Leverkusen, Germany). The animals were then fixed on a styropore plate with their ventral side facing the irradiation source and irradiated with 10 Gy (at 175 kV and 15 mA for about 10 minutes) using a Gulmay RS225 X-ray system (X-Strahl). 10 Gy dose was used because this dose has been applied in palliative radiotherapy.25 The night experiments were performed under dim red light.

2.4 | Locomotor activity

All recording experiments were performed in light and sound proof cabinets with automatic control of the photoperiod (lights on/off) (Scanbur, Germany). The light conditions were kept constant during the whole experiment with light intensity during the light phase was 300 lx. Animals were adapted to the experimental conditions at least two weeks before the experiments. Mice were housed individually in cages equipped with infrared movement detectors linked to an automated recording system (Mouse-E-Motion, Hamburg, Germany). Spontaneous locomotor activity was continuously recorded during the entire experiment in 10-minutes intervals. The actograms, chi-squared periodograms, activity profiles, and the relative power of phase (fast Fourier transformation; FFT) were analyzed by Clocklab software (Actimetrics). In addition, median of activity (MOA) which is defined as time point at which the mouse has achieved 50% of its daily activity was used to determine the chronotype. The variance of MOA is a measure for instability of the chronotype.16,26

2.5 | Blood collection and corticosterone measurement

In parallel groups, blood was collected from 8-month-old animals of the tumor (20 animals, n = 5/time point), phenobarbital (12 animals, n = 3/time point), and control (20 animals, n = 5/time point) groups at four different ZTs (ZT02, ZT08, ZT14, and ZT20). In the tumor + irradiation group, 20 HCCs bearing mice were randomly divided into 4 subgroups (n = 5/time point) for irradiation with a dose of 10 Gy at the four different ZTs, that is, ZT02, ZT08, ZT14, and ZT20. 48 hours later, the animals were sacrificed at the same ZTs used for irradiation, quickly dissected and the blood was withdrawn from the right atrium in serum separator tubes, allowed to clot for 20 minutes and then centrifuged at 1000×g at 4°C for 10 minutes to separate the serum. Serum was aliquoted, snap frozen in liquid nitrogen, and stored at −80°C until being assayed. Sampling during the night was performed under dim red light. Serum corticosterone levels were measured using an enzyme-linked immunosorbent assay (Corticosterone ELISA Kit #ab108821, Abcam, UK) following the manufacturer’s instructions and using the microplate photometer Multiskan FC (Thermo scientific).

2.6 | Control for effects of short-term anesthesia

To control the effects of short-term anesthesia, locomotor activity was recorded in HCC bearing mice for 7 days and then the animals were deeply anesthetized by intraperitoneal injection of a mixture of ketamine (100 mg/kg, Inresa) and xylazine (10 mg/kg, Rompun 2%, Bayer Leverkusen, Germany) at ZT20. Thereafter, the locomotor activity was recorded for 5 consecutive days and all locomotor activity parameters were evaluated as mentioned above.

To control the effects of short-term anesthesia on corticosterone levels, the animals were deeply anesthetized as mentioned above at ZT20. Another group of animals were left without injection (control group). 48 hours after ketamine injection, the control and the injected animals were sacrificed at the same ZT, quickly dissected and the blood was withdrawn and processed as mentioned above, and the corticosterone levels were evaluated.

2.7 | Animal perfusion and immunohistochemistry

Immunohistochemistry was performed with 12 animals of the tumor, tumor + irradiation, phenobarbital, and control groups. The animals were sacrificed at four different ZTs: ZT02, ZT08, ZT14 and ZT20 (n = 3/time point). All animals of the tumor and tumor + irradiation groups had either single or multiple tumors. The animals were anesthetized by intraperitoneal injection of a mixture of ketamine and xylazine as mentioned above and then perfused transcardially with 0.9% NaCl followed by 4% paraformaldehyde (PFA) in 0.1 M phosphate-buffered saline (PBS, pH 7.4). Perfusion during the night was performed under dim red light. Brains were removed from the skull, postfixed in 4% PFA for 24 hours, and then cryoprotected in 30% sucrose for another 24 hours. Coronal brain sections (30 μm thick) were prepared on a cryostat (Leica CM, Germany). Sections were washed with PBS.
containing 0.2% Triton-X 100 (PBST) and treated with 0.6% H₂O₂ for 30 minutes to block endogenous peroxidase. To reduce nonspecific staining, sections were blocked for 1 hour with 5% normal goat serum in PBST, then incubated overnight at 4°C with p-ERK (1:1000, monoclonal rabbit anti p-ERK, Cell Signaling Technology, Frankfurt am Main, Germany). On the next day, sections were incubated with biotinylated goat anti-rabbit IgG (1:500, Thermo Scientific) diluted in blocking buffer and then incubated with VECTASTAIN® Elite® ABC solution (1:200, Vector Laboratories) in PBST for 1 hour. This was followed by incubation with 0.05% 3, 3′-diaminobenzidine (DAB) (1:100, Sigma Aldrich, USA) for 5 minutes. Slides were cover-slipped using DePeX (SERVA Electrophoresis).

### Data acquisition

Images were acquired using BZ-X800 series microscope (Keyence, Japan) with a 20× objective in bright field mode. All images were processed using constant settings. p-ERK immunoreactive cells in the SCN were quantitatively analyzed using Image J software (http://rsbweb.nih.gov/ij) as previously described.²⁷
2.9  Statistical analysis

Statistical analysis was performed by using GraphPad Prism 8 software. Data are expressed as mean ± standard error of the mean (SEM). Paired t-test was used to estimate the significant differences between two groups. Comparison between more than 2 groups was performed using ordinary one-way analysis of variance (ANOVA) followed by Tukey’s test for multiple comparisons. Two-way analysis of variance (ANOVA) was used to examine differences according to time and groups followed by Sidak’s test for multiple comparisons between groups. The results were regarded as significant at P < .05.

3  RESULTS

3.1  Effects of phenobarbital and tumor on spontaneous locomotor activity rhythms

Animals of control, phenobarbital, and tumor (before irradiation) groups showed a higher activity during the dark phase as compared with the light phase (Figure 1 and Figure 2). However, in the phenobarbital group, the activity during the light phase was significantly increased as compared with the control group (Figure 2). In the tumor group, the total activity especially the activity during the dark phase was significantly reduced as compared with the phenobarbital group (Figure 2).

Periodograms (Figure 3A) and FFT (Figure 3B) revealed that the relative power of phase was significantly decreased in the phenobarbital group as compared with the control group (P < .05). There was no difference in the relative power of phase between the tumor and phenobarbital groups (P > .05, Figure 3B).

Neither the chronotype (MOA) (Control: n = 5, 20.08 ± 0.6; Phenobarbital: n = 5, 19.9 ± 0.6; Tumor: n = 20, 20.5 ± 0.3) nor the variance of MOA was different among the three groups (P > .05, Figure 3B).

3.2  Effects of irradiation on spontaneous locomotor activity rhythms

Locomotor activity profiles of mice with HCC (tumor group) were compared before and after irradiation at different ZTs. Before irradiation, all mice showed a higher activity during the dark phase as compared with the light phase (Figure 1 and Figure 4). However, after irradiation, the difference in activity during the light and the dark phase disappeared (Figure 4), except for the mice which were irradiated at ZT20 (Figure 4D). Moreover, the total activity especially the activity during the dark phase was significantly decreased after irradiation (Figure 4).

The irradiation did not affect the chronotype (Table 1). However, periodograms and FFT revealed that the relative power of phase was significantly reduced in mice which received irradiation at ZT20 (Figure 5). Moreover, in this subgroup, the variance of MOA was also significantly increased (Figure 5B).

Short-term anesthesia on its own does not elicit any effect on the spontaneous locomotor activity rhythms (Figure S3A).

3.3  Effects of phenobarbital, tumor, and irradiation on serum corticosterone levels and SCN activity

The control group showed daily changes in serum corticosterone levels with a peak at ZT14 (2 hours after light off). In the phenobarbital group, this peak was blunted. The tumor group before irradiation showed an additional peak in serum corticosterone at ZT08. In mice irradiated at ZT08 and ZT20, the corticosterone levels were significantly increased as compared with the tumor group (Figure 6). Short-term anesthesia on its own does not elicit any effect on the corticosterone levels (Figure S3B).

In the control group, the number of p-ERK immunoreactive cells was higher at ZT02 and ZT08 as compared with ZT14 and ZT20. In the phenobarbital group, the number of p-ERK immunoreactive cells was also higher at ZT02 and ZT08 as compared with the time points during the dark phase and significantly higher as compared with the respective time points in the control group. In the tumor group before irradiation, the number of p-ERK immunoreactive cells showed a peak at ZT02, but at ZT08, the number of p-ERK immunoreactive cells was significantly reduced as compared with the tumor group (Figure 6).
immunoreactive cells was significantly reduced as compared with the phenobarbital group.

Irradiation of the animals did not affect the number of p-ERK immunoreactive cells at all irradiated ZTs except at ZT20 at which the number of p-ERK immunoreactivity was significantly higher ($P < .001$) as compared with the HCC bearing mice before irradiation (Figure 7).

**DISCUSSION**

The primary aim of our study was to analyze how tumor development and radiotherapy at different times of the day affect the circadian system. As readouts for a functional circadian system, rhythms in spontaneous locomotor activity and serum corticosterone levels were determined before and after therapeutic irradiation. In addition, p-ERK immunohistochemistry was used as a marker for rhythmic SCN neuronal activity under light/dark conditions.28-30

**4.1 Impact of chronic phenobarbital treatment**

The mice treated with phenobarbital showed a higher spontaneous locomotor activity during the dark phase as compared with the light phase and a period length close to 24 hours. This indicates that phenobarbital does not affect the response of the circadian system to light per se. However, the activity counts during the light phase was significantly increased as compared with the control mice, reminiscent of sleep disruption. In nocturnal rodents, light suppresses locomotor activity.31 Thus, an increased locomotor activity during the light phase suggests a reduced effect of light or an increased agitation during the sleep phase, which overrides the suppressive effect of light. Our findings are in agreement with studies by Forcelli et al32 and Quinlan et al33 who reported that administration of phenobarbital in the sensitive postnatal period can cause impairments in the behavior of rodents including abnormalities in locomotor activity and
social behavior which become obvious in later life. In addition, patients treated with anticonvulsant drugs including phenobarbital suffer from sleep disruption. One side effect of chronic phenobarbital treatment is the reduction in GABA receptor expression. GABA is known as a potent inhibitory neurotransmitter which plays an important role in the sleep-wake cycle. Presynaptic GABA receptors, which mediate the transfer of light information to retinal ganglion cells, could also be affected by chronic phenobarbital treatment. However, as p-ERK immunoreaction was highly rhythmic in the phenobarbital group, a severe corruption of the light input pathway is unlikely.

The relative power of phase was significantly decreased in the phenobarbital group as compared with the control group. This parameter reflects how much of the activity rhythm is due to the 24 hours component and thus reflects rhythm stability. Consistently, the daily variation of serum corticosterone was damped in the phenobarbital group. Phenobarbital affects the amplitude of time-of-day dependent rhythms of locomotor activity and corticosterone levels presumably distal to the SCN as p-ERK-immunoreaction was highly rhythmic. The levels of corticosterone during the light/inactive phase were not increased upon phenobarbital treatment. This conforms to a study in humans which reported that acute and chronic phenobarbital treatment does not alter the cortisol levels during the night/inactive phase.

4.2 Impact of HCC development

The mice which developed HCC showed a higher spontaneous locomotor activity during the dark phase as compared...
HASSAN et al.

but also abnormally increased during the light/dark phase and a period length close to 24 hours. This indicates that tumor development does not affect the response of the circadian system to light per se. In comparison with the phenobarbital treated mice, the total activity especially the activity during the dark phase was significantly reduced, reminiscent of fatigue. This is consistent with the results of Verma et al\(^3\) who reported that mice with HCC showed alterations of the locomotor activity rhythm. Curiously, in contrast to a disruption of rhythmic locomotor activity in mice with chemically induced HCC,\(^3\)\(^9\) rhythmic locomotor activity was not affected in mice injected with a human HCC cell line.\(^4\)\(^0\) However, it is unknown how the different methods of tumor induction could impose different effects on the circadian system. In the tumor group, the relative power of phase was as low as in the phenobarbital group; thus, tumor development had no additional effect on rhythm stability.

In contrast to the phenobarbital group, the corticosterone levels in the tumor group were significantly increased during the early activity phase as in the control group (ZT14) but also abnormally increased during the light/inactive phase (ZT08). This increase in corticosterone levels during the inactive phase might reflect an increase in chronic stress and/or neuroinflammation (see below). Interestingly, the immunoreaction for p-ERK in the SCN at ZT08 is significantly reduced in the tumor group as compared with the phenobarbital group. Although glucocorticoid receptor signaling acts by altering the activity of various kinases including p-ERK and impinges on circadian regulation,\(^2\)\(^1\) little is known on the direct effects of stress on p-ERK expression in the SCN.

Severe sleep problems, disrupted and decreased locomotor activity rhythm and decreased life quality, which are associated with CRF, were reported in HCC, breast and metastatic colorectal cancer patients.\(^1\)\(^-\)\(^3\) Moreover, alterations of the glucocorticoid rhythm and its increase at sleep time were observed in HCC and ovarian cancer patients and this was closely related to poor sleep quality and fatigue.\(^2\)\(^4\)\(^1\) In a cross-sectional study, the elevation in the cortisol level at the rest phase was shown to be related to short sleep duration and high sleep disturbance suggesting a direct relation between both rhythms.\(^4\)\(^2\) Patients with liver metastasis of colorectal cancer had increased levels of the proinflammatory cytokines IL-6 and transforming growth factor-alpha (TGF-\(\alpha\)) which coincided with increased fatigue and disturbances in the cortisol and rest/activity rhythms.\(^3\)

**4.3 Impact of radiotherapy**

Over 50% of cancer patients who undergo chemo- or radiotherapy suffer from CRF as well as sleep problems.\(^4\)\(^5\) Breast cancer patients subjected to radiotherapy tended to have more sleep disturbance and longer sleep latency than breast cancer patients who did not receive treatment.\(^1\) Administration of cancer therapies at the right time of day can minimize the circadian alterations and improve the life quality.\(^4\)\(^3\) However, little is known about the effect of radiotherapy on rest/activity and glucocorticoid rhythms if administered at different time points during the day. In our study, mice bearing HCC were irradiated at four different ZTs (ZT02, ZT08, ZT14, and ZT20) to investigate the short-term effect of radiotherapy on the locomotor activity rhythm and corticosterone levels.

Total locomotor activity especially activity during the dark phase was significantly decreased after irradiation with 10 Gy, irrespective of the time of irradiation. Moreover, the difference between activity counts during the light and the dark phase was abolished after irradiation, except after irradiation at ZT20. However, specifically after irradiation...
Radiotherapy is the common cause of the HPA axis dysfunctions in cancer patients. The additional stress induced by radiotherapy further heats up the proinflammatory response leading to a vicious circle. Rats exposed to 1-10 Gy irradiation showed a significant increase in the plasma corticosterone levels as compared with non-irradiated rats. Consistently, in this study, the serum corticosterone levels were significantly increased in tumor + irradiated group as compared with the tumor group, especially after irradiation at ZT08 and ZT20 reflecting increased chronic stress and/or neuroinflammation. The number of p-ERK immunoreactive cells in the SCN was significantly increased in mice which received radiotherapy at ZT20. This increase at ZT20 is unexpected as p-ERK in the SCN is induced by light, but supports our hypothesis that stress related glucocorticoid receptor signaling affects p-ERK expression in the SCN and subsequent locomotor activity rhythm stability.

In conclusion, irradiation strongly affects locomotor activity rhythm and corticosterone levels but the timing of administration makes small but significant differences. The corticosterone levels were lowest after irradiation at ZT02 which reflects less stress. Moreover, at this time point, no effects were observed on the rhythm stability and the number of p-ERK immunoreactive cells. In contrast, the effect on rhythm instability was highest at ZT20 consistent with a significant increase in the corticosterone levels which reflects more stress. Thus, in order to minimize stress and disruption of the circadian system, it appears that radiotherapy should be applied at the beginning of the rest phase, but not at the end of the active phase, at least in the HCC mouse model investigated here. Translational studies are now required to clarify whether these findings in night-active animals can be confirmed in day-active humans and to test whether also for patients suffering from HCC irradiation at the beginning of the rest phase minimizes stress and disruption of the circadian system.

ACKNOWLEDGEMENTS
S. A. H. is supported by German Egyptian Research Long-term Scholarship (GERLS) Program of the DAAD. We acknowledge support by the Heinrich-Heine-University Düsseldorf for open access funding. We thank Ralf Fassbender, Hanna Bellert, Angelika Hallenberger, and Ursula Lammersen for excellent technical support.

CONFLICT OF INTEREST
The authors have no conflict of interest.

AUTHOR CONTRIBUTION
Soha A. Hassan involved in concept/design, acquisition, analysis and interpretation of data, and drafting of the manuscript. Amira A. H. Ali involved in acquisition, analysis, and interpretation of data. Mona Yassine involved in acquisition and analysis of data. Dennis Sohn involved in acquisition of data. Martina Pfeffer involved in data analysis/interpretation. Reiner U. Jänicke involved in critical revision of the manuscript. Horst-Werner Korf involved in concept/design, acquisition and interpretation of data, supervision, and drafting of the manuscript. Charlotte von Gall involved in data interpretation and drafting of the manuscript. All authors have read and approved the manuscript.

DATA AVAILABILITY STATEMENT
The data that support the findings of this study are available from the corresponding author upon reasonable request.

ORCID
Horst-Werner Korf  https://orcid.org/0000-0002-5051-0303

REFERENCES
1. Fortner BV, Stepanski EJ, Wang SC, Kasprowicz S, Durrence HH. Sleep and quality of life in breast cancer patients. J Pain Symptom Manage. 2002;24(5):471-480.
40. Huang A, Bao B, Gaskins HR, et al. Circadian clock gene expression regulates cancer cell growth through glutaminase. *Acta Biochim Biophys Sin (Shanghai)*. 2014;46(5):409-414.

41. Weinrib AZ, Sephton SE, Degeest K, et al. Diurnal cortisol dysregulation, functional disability, and depression in women with ovarian cancer. *Cancer*. 2010;116(18):4410-4419.

42. Kumari M, Badrick E, Ferrie J, Perski A, Marmot M, Chandola T. Self-reported sleep duration and sleep disturbance are independently associated with cortisol secretion in the Whitehall II study. *J Clin Endocrinol Metab*. 2009;94(12):4801-4809.

43. Ortiz-Tudela E, Iurisci I, Beau J, et al. The circadian rest-activity rhythm, a potential safety pharmacology endpoint of cancer chemotherapy. *Int J Cancer*. 2014;134(11):2717-2725.

44. York JM, Blevins NA, Meling DD, et al. The biobehavioral and neuroimmune impact of low-dose ionizing radiation. *Brain Behav Immun*. 2012;26(2):218-227.

45. Lira FS, Esteves AM, Pimentel GD, et al. Sleep pattern and locomotor activity are impaired by doxorubicin in non-tumor-bearing rats. *Sleep Sci*. 2016;9(3):232-235.

46. Firoozi M, Besharat MA. Cortisol-a key factor to the understanding of the adjustment to childhood cancer. *Iran J Cancer Prev*. 2013;6(1):1-7.

47. Schmiegelow M, Feldt-Rasmussen U, Rasmussen AK, Lange M, Poulsen HS, Muller J. Assessment of the hypothalamo-pituitary-adrenal axis in patients treated with radiotherapy and chemotherapy for childhood brain tumor. *J Clin Endocrinol Metab*. 2003;88(7):3149-3154.

48. Crews FT, Sarkar DK, Qin L, Zou J, Boyadjieva N, Vetreno RP. Neuroimmune Function and the Consequences of Alcohol Exposure. *Alcohol Res*. 2015;37(2):331-351.

49. Kandasamy SB, Thiagarajan AB, Harris AH. Possible involvement of prostaglandins in increases in rat plasma adrenocorticotrophic hormone and corticosterone levels induced by radiation and interleukin-1 alpha alone or combined. *Fundam Appl Toxicol*. 1995;25(2):196-200.

50. Cohen EP, Bruder ED, Cullinan WE, Ziegler D, Raff H. Effect of high-dose total body irradiation on ACTH, corticosterone, and catecholamines in the rat. *Transl Res*. 2011;157(1):38-47.

51. Coogan AN, Piggins HD. Circadian and photic regulation of phosphorylation of ERK1/2 and Elk-1 in the suprachiasmatic nuclei of the syrian hamster. *J Neurosci*. 2003;23(7):3085-3093.

52. Obrietan K, Impey S, Storm DR. Light and circadian rhythmicity regulate MAP kinase activation in the suprachiasmatic nuclei. *Nat Neurosci*. 1998;1(8):693-700.

**SUPPORTING INFORMATION**

Additional supporting information may be found online in the Supporting Information section.

**How to cite this article:** Hassan SA, Ali AAH, Yassine M, et al. Relationship between locomotor activity rhythm and corticosterone levels during HCC development, progression, and treatment in a mouse model. *J Pineal Res*. 2021;70:e12724. [https://doi.org/10.1111/jpi.12724](https://doi.org/10.1111/jpi.12724)