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Seasonal Variation of Circadian Activity of Rat Measured by Circadian Locomotor Modified Cage

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ABSTRACT: *Circadian rhythm variations are related to seasonal photoperiodic variations, which affect animal as well as human physiology and behavior. The present study aimed to evaluate the effect of photoperiodic variations on circadian locomotor activity using circadian locomotor modified cages to maintain animals' welfare demands. Twelve male Sprague Dawley rats (140-180g) housed under natural photoluminous periods. Rats were divided into two groups (each, n=6), summer group (Long photoperiod; GA; LD 14:10) and winter group (Short photoperiod; GB; LD 11:13). Circadian locomotor activity rhythm was examined in both groups. Rats were housed individually in circadian locomotor plastic cages with voluntary running wheel. Results revealed that GB rats had significantly ($p<0.05$) greater locomotor activity during the day and longer tau than GA rats. Locomotor activity of GB significantly ($p<0.05$) decreased compared to GA. Taken together, it could be concluded that the ability of GA rats to entrain to long photoperiod compared to GB, suggests that the photoperiod affects the circadian locomotor rhythm. Moreover, the present study tried to introduce a design for circadian locomotor modified cage.*

Keywords: Circadian, photoperiod, locomotor, running wheel, rats.

I. INTRODUCTION

Circadian homeostasis regulation is organized by an endogenous biological clock located in the suprachiasmatic nuclei (SCN) of the hypothalamus. The circadian clock is entrained by light information that travels directly from light-sensitive ganglion cells in the retina to the SCN, thereby synchronizing individuals' physiology and behavior from the external day-night cycle (Schroeder *et al.*, 2002). In temperate zones duration of daylight (photoperiod) changes with the seasons, which affects animal as well as human physiology and behavior (Elliott and Tamarkin, 1994). Whereas, most mammals respond seasonal photoperiod changes with altered physiology and behavior. Photoperiodic information from the environment is conveyed to organism by a circadian rhythm of melatonin production in pineal gland (Sumova *et al.*, 1995). The resulting alteration in the duration of the nocturnal melatonin signal, compressed during long summer days and decompressed during short winter days, that was appearing as serve as an endogenous photoperiodic message. Circadian locomotor activity behavior test by voluntary running wheel has many characteristic agents as not stressful, familiar environment for subject, long-term observation of circadian activity rhythm, easy to perform tests, no animal handling and is applicable to human disease models (Canini *et al.*, 2009). Volunatry running wheel test is sensitive for rodents that may reduce depression-like signs in them from chronic stress (Tsuchida *et al.*, 2009). Otherwise, rats and mice are most commonly used laboratory animals in experimental research (Mering, 2000). They spent part of their life in home cages, so, laboratory animals' house has mainly been designed to meet economic and ergonomic demands for animal welfare (Baumans, 2005). In combination with control of environmental and health monitoring factors, economy and management has led to the development of housing systems for laboratory animals that are easy to handle, clean and store, inexpensive and/or make it possible to keep many animals on a restricted area (Baumans, 2005). The present manuscript designed circadian locomotor modified cage with maintaining the animals' welfare conditions and highlight the impact of photoperiod duration on the circadian locomotor activity of rats during summer (long photoperiod) and winter (short photoperiod) seasons.

II. MATERIALS AND METHODS

Animals: Twelve male Sprague Dawley rats (140-180g) were used for the experimental procedures. Rats were obtained from the breeding unit of animal house in Zoology Department, Faculty of Science, Suez Canal University, Egypt, under (temperature 26ºC, natural light/dark cycles; LD) conditions and allowed free access to food and water. They were maintained for one week in the experimental room for habituation. Rats were divided into two groups (each, n=6), summer group (Long photoperiod; GA; LD 14:10) and winter group (Short photoperiod; GB; LD 11:13). They were housed individually in plastic circadian locomotor cage under natural circadian and seasonal luminosity 07:00 am - 21:00 pm for long photoperiod and 08.00 am -18:00 pm during short photoperiod.

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Cage design: Six plastic cages were used for the experimentation consisting of two parts. The upper part oblong plastic lid with stainless steel wire mesh (45x28x8cm). The lower rectangular plastic part (45x28x25 cm) with mesh-flooring and drawer sheet for a bedding change as shown in figure 1. External water bottles and food bowls were used to avoid direct handling of animals and thereby minimize stress and disturbance during the experimental period. Running wheel of the experimental animal was placed individually in each cage to record the circadian locomotor activity rhythm. Running wheel with radius of about 9.5cm and a width of 4cm which placed at the center of each cage (Fig. 2A). The motor activity was recorded continuously through the experimental periods.

Data collection: Locomotor activity was automatically monitored by emitted infrared switch (photocell) where every interruption caused by rats was registered. Photocell was fixed at about 5cm from the floor of each cage outside the cage and was covered by a piece of black plastic (Fig. 1). Animal's movement was converted into accounts by a mirror piece attached to the running wheel as when the mirror piece interrupted the infrared light beam, it reflects the light beam to adjacent infrared sensor. The photocells which were attached to a circuit for counting these interruptions. Photocell fixed outside the cages directly in front of running wheel (Fig. 1). Photocell circuits were attached to USB converters and power supply. A converter USB attached by a multiport serial (model Intopic, USB 2.0, 4 ports) that was attached to computer hardware to give digital serial data from all circuits (Figs. 2B,3).

Data Analysis: Data from individual cage were acquisited by specially designed program "Complay", where channel numer, sampling rate and total file size could be adjusted. Such data were better visualized and graphed with actogram program software (Actogram j). However, single-channel files were analyzed using a general spreadsheet Excel program (Excel 2010; Microsoft 7). Actograms provide a graphic illustration of the daily patterns of running-wheel activity (Schmid *et al.*, 2011). Double-plotting is especially helpful to visualize non-24hr rhythms (Fig. 6). A periodogram is constructed from a spectral analysis the running wheel activity over time. Periodograms show the relative power for a range of pre-set periods, and are commonly used to determine the free-running period. The time onset of running-wheel activity (tau; τ) method was calculated manually as mentioned in Schmid *et al.* (2011).

Statistical analysis: Locomotor activity is represented as mean±SE of running wheels' counts at 6-hours intervals (n=6 rats/season). The mean values of four times intervals in each group and between two groups were compared using one-way ANOVA followed by Post hoc Tukey test (IBM SPSS statistic 21 software). Differences were considered significant at $p<0.05$ level.

III. RESULTS

The current study succeeded to design a reliable and precise set up for long-term recording of circadian locomotor activity rhythm in the rats. Rats activity was increased during the active phase (night) by the beginning of the darkness, then decreased during the rest phase (day) by onset of the light as shown in figure 5. Also, in two photoperiod groups, specific differences were observed in activity patterns. Representative rhythms from individual rats with circadian patterns are shown in figure 5.

During the present study, the locomotor activity of GA rats in long photoperiod (LD 14:10) activated at the same time each day, soon after the period of lights were turned off as shown in double-plotted actogram on the left part (fig. 5A). Priodogram on the right shows a strong peak at 24.2hr, also τ from 23.89 to 24hr, consistent with entrainment to a precise 24hr LD cycle. The locomotor activity reached its acrophase (373.3±32 counts/ 6-hour intervals) around 21:00. Meanwhile, it reached its trouph (70±25counts/ 6-hour intervals) around 09:00. As shown in figure 4.

Furthermore, in the current study, figure 5B illustrated the locomotor activity of GB rats that were maintained during short photoperiod (LD 11:13). In this case, the daily onset of locomotor activity occurred slightly later each day, creating a rightward drift activity. According to the periodogram, maximum power is observed at 26.15hr and τ of right ward drift activity from 25.7 to 26hr. The high level of locomotor activity in GB (191.3±26 counts/ 6-hour intervals) was reached around 03:00 and its trouph (86.3±14 counts/ 6-hour intervals) around 15:00. Locomotor activity of GB rats significantly ($p<0.05$) decreased after maintained in short photoperiod compared to GA maintained in long photoperiod, as shown in figure 4.

IV. DISCUSSION

In the present study, modified new circadian locomotor activity plastic model cages were designed to record locomotor activity of rats under natural LD cycles. Furthermore, the circadian locomotor activity of rats differed between summer and winter seasons was observed, regarded to photoperiod length.

Plastic cage materials are easy to clean and allow proper inspection of animals, additionally these cages were used for the cage body construction, since stainless steel had inherent difficulties (Hall *et al.*, 2000). In the present study, handmade plastic cages were used due to their advantage of filtering out harmful glare, allowing rats to hide from humans and neighboring rats, well preserving heat in solid plastic cage and allowing observation of rats from outside the cage (Hargreaves, 2000). Construction and management of rats' cages were determined to a large extent how environmental factors, such as temperature, light levels, humidity and air quality impact of the rat (Hall *et al.*, 2000).

In the current work, plastic circadian locomotor cage with wire mesh floors were used to reduce the contact with the animal as mentioned by Cain *et al*. (2004). Whereas, animal handling as well as changes in cages or bedding can all have effects on circadian rhythms (Cain *et al.*, 2004). Ashoka Deepananda (2013) reported that laboratory animals should be reared in a comfortable cage with a sufficient floor type in order to ensure better welfare and refered to avoid the use of grid floor cages to the best results from the animal experimentation. Otherwise, in the present work, height of the cages were 25cm to allow rats stand on their hind legs and stretch up fully. The maximum height achieved by rats during upright standing is about 26-30cm. Part of the normal behavior of rats is standing on their hind legs and stretch upright for periods to talling 2hrs per day for food and water. If the height > 30cm leading to develope bony and cartilaginous damage of the femoral heads (Ashoka Deepananda, 2013).

Furthermore, the present findings suggest the recorded setup by using voluntary running wheels and infrared photocell switch enabled us to record the animal's circadian locomotor activity patterns automatically in plastic cage without upsetting the normal pattern of its behavior. The computerized monitoring system also permits continuous long-term monitoring of the animals activity. The obtained results of the locomotor activity after one week habituation in natural LD cycle, clarified that rats revealed typical circadian locomotor activity rhythms as nocturnal animals by low activity in subjective day and high activity in subjective night as reported with Paulus *et al.* (1999).

Otherwise, in the present study, GA rats showed regular and steady activation in long photoperiod (LD 14:10) with shorter duration activity. Per contra, GB rats showed irregluar and patchy activation in short photoperiod (LD 11:13) with long duration activity. These result accompanied with Elliott and Tamarkin (1994). Warner *et al.* (2010) reported short photoperiod in winter season caused loss of circadian locomotor activity patterns and amplitude. During the siting study, GA rats illustrated entramintent with short duration in long light summer accompanied to increased locomotor activity. While, GB rats re-entrained with long duration in short light winter accompanied to significantly ($p<0.05$) decreased in locomotor activity, that agreed with Malpaux *et al.*(2001).

In addition, during the present result, GB rats in short photoperiod (LD 11:13) illustrated rightward drift periods of greater than 24h are expected to reentrain faster to phase shift of locomotor activity (Fig. 5B). Phase shift of GB locomotor activity by 6hrs that was observed in figure 4, may be due to expansion of the dark period increased the duration of locomotor activity (Samantha, 2011), or may be following the rhythmic production of the pineal hormone melatonin, according to seasonal variations, which is shorter on long photoperiod than on short photoperiod (Malpaux *et al.*, 2001). Also, phase shift of GB locomotor activity may be due to CLOCK genes transcription of period 1 and 2 genes (*Per1* and *Per2*). Whereas, photoperiod length promote transcription of Per genes for phase shift (Malpaux *et al.*, 2001) in SCN.

V. CONCLUSION

To sum up, ability of GA rats to entrain to long photoperiod compared to GB, suggests that the photoperiod affects the circadian locomotor rhythm through the rat SCN or melatonin rhythm. Hence, the whole central timekeeping mechanism within the rat circadian clock measures not only the daytime but also the time of the year, i.e. the actual season. Moreover, laboratory animals should be housed in circadian locomotor cages with comfortable materials types in order to provide better welfare to receive the best results from the animal experimentation.

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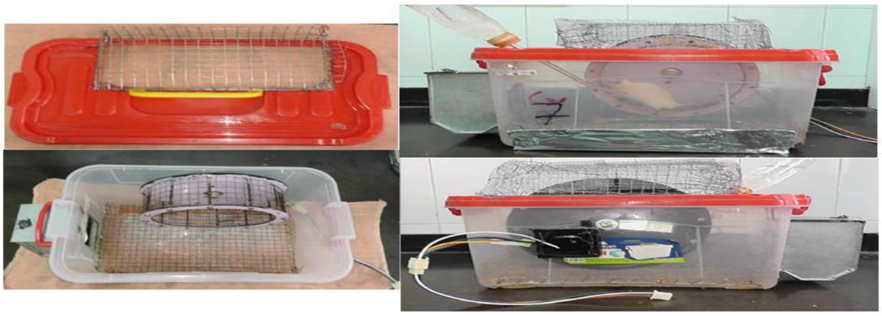

Fig. 1: Design of experimental cage for locomotor activity recording illustrating the cage components

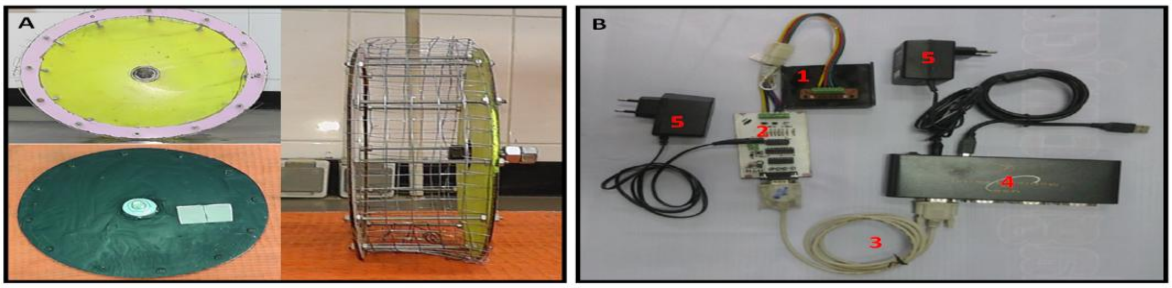

Fig. 2: Voluntary running wheel and a circuit for counting, including: 1) Photocell sensor, 2) photocell circuit, 3) Converter, 4) Multi-portal serial, 5) power supplies.

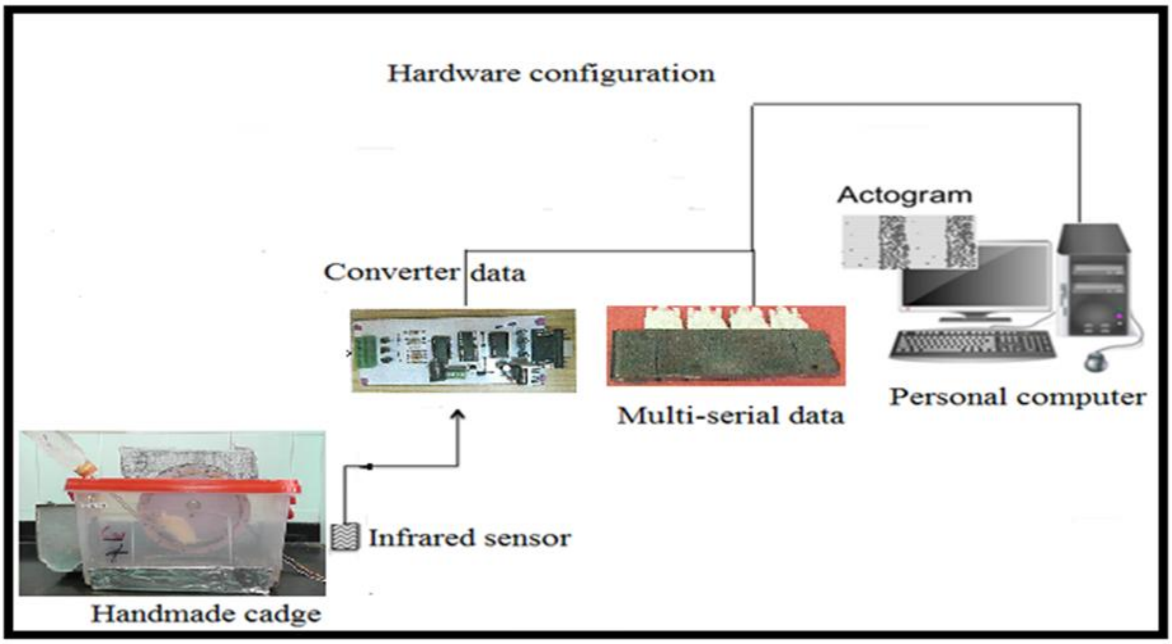

Fig. 3: Hardware configuration of locomotor system

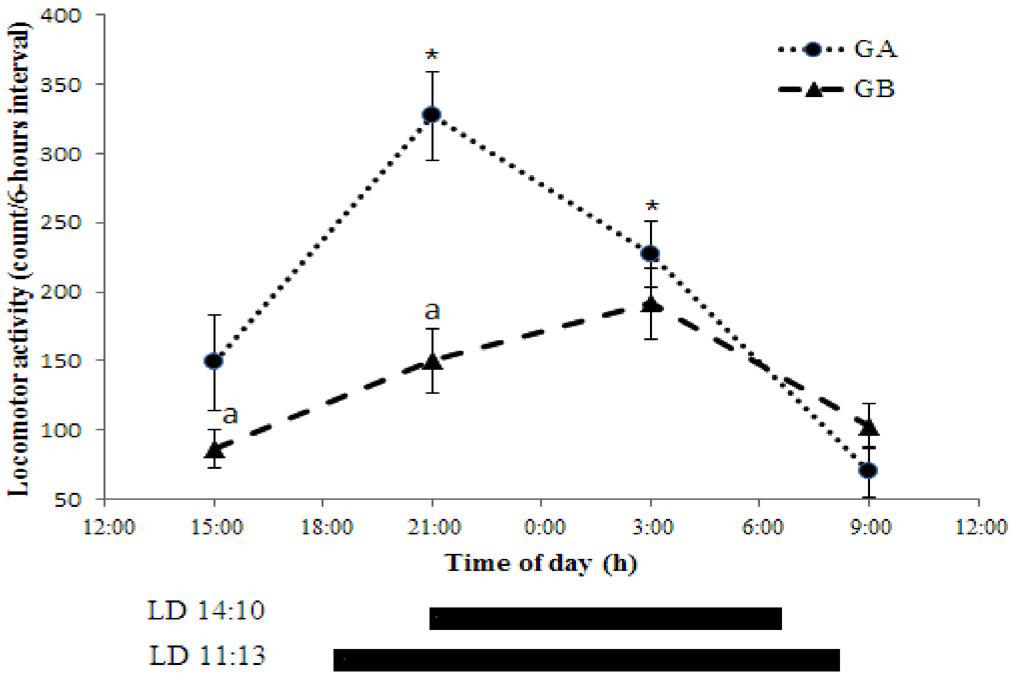

Fig. 4: Circadian locomotor activity rhythms of rats during long photoperiod LD 14:10 (Summer season) and short photoperiod LD 11:13 (Winter season). Dark bars refer to the natural night phase. Locomotor activity is represented as mean±SE of running wheels' counts at 6-hours intervals (n=6 rats/season). Data was analyzed by one-way ANOVA test followed by Tukey post hoc test. Statistical significance is indicated at $p<0.05$ as following, *: comparison inside each group, a: comparing GB vs GA groups.

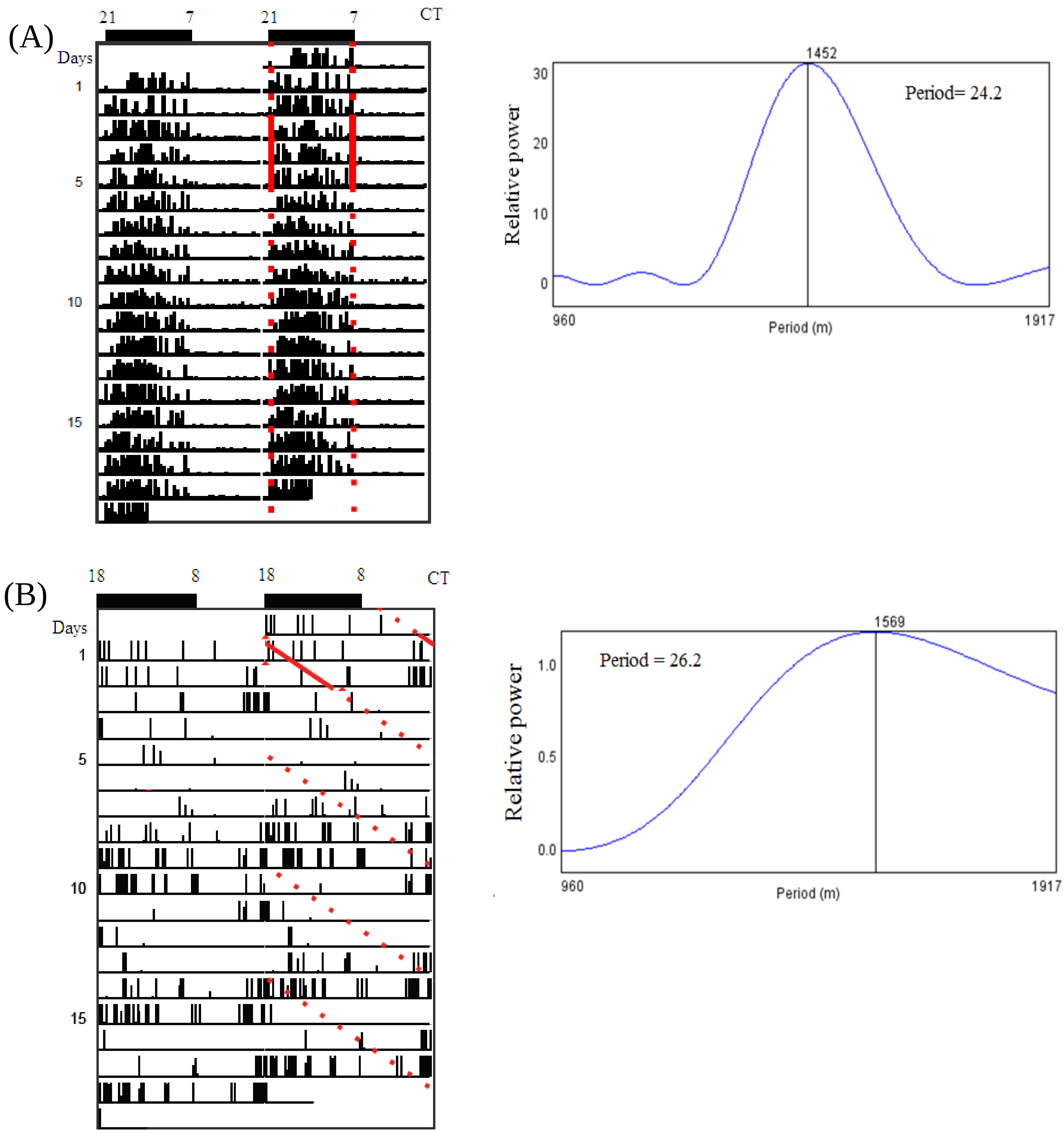

Fig. 5: Running wheel activity of rats housed in a natural LD cycle that representative by actograms and periodograms (GA) in long photoperiod, LD 14:10 and (GB) short photoperiod, LD 11:13 (each, n=6). Figure 5A illustrates the rat's activity in long photoperiod. Figure 5B illustrates the rat's behavior in short photoperiod. Double-plotted actograms on the left part illustrate lighting conditions along the top, 48hr of running-wheel activity along the X-axis, and plot sequential days from top to bottom. Periodograms on the right part perform a spectral analysis on the running wheel data illustrated in the actograms. CT: Circadian Time. Red lines: the onset and offset of duration of rat's activity.