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Circadian Patterns of Rats in Their Home Cages Detected Using a Video Tracking System

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Abstract

The diurnal rhythm is the common event in nature and specially shows in the behavioral patterns. Using the infrared sensor or photo beam detector to detect this 24-h rhythmicity in behaviors of mammalian, including in the rats and mice, is also the common way. The photo-sensory detecting mean is friendly and its advantage is unrestricted by light density and light-dark transition. However, this kind of equipment is cost-expensive and uneasy to fit for home cage in rodents. In this study, we tried to use the video-tracking system to detect the rhythmic activity of rats in their home cages. Adult male Sprague-Dawley rats, weighing 250-280 g, were used in this study and individual was kept in its own cage. Combined with the infrared sensitive charge-coupled device (CCD) camera and with automatically lights-off sensitive infrared illuminants as the accessory device, we found that animals exhibited the circadian locomotor activity in either light-dark cycles or constant darkness conditions. Moreover, the rhythmic patterns of locomotion in animals were affected by the one-hour exposure of white light under the constant darkness condition. The phase-advanced effects were found by the video tracking system. In summary, the video tracking system is the useful way to detect the rhythmic activity, especially in long-term circadian rhythmicity, in rats.

Key Words: circadian rhythm, locomotion, path analyzer, phase shifting, recording

Introduction

Traditionally the common ways to get the behavioral related data by scientists are the observational/manual way (1), and the semi/fully automatically one (15). The former advantage is that the pattern or qualitative profile of behavior can be observed directly. Although the well-trained observers enable to monitor more than one animal in a group of animals at the same time, the observational and manual way is still time-consuming, labor intensive and is needed the well trained. The other problem for observational/manual way is not suitable for the long term and continual study, such as the monitoring of behaviors related to the circadian rhythmicity. Thus the semi/fully automatically way to monitor the animals' behaviors is to develop and overcome the disadvantages of observational/manual way. The most common and automatic method for recording the animal's locomotor activity is by the photocell or infrared beams. Additionally the chronobiologists also use the running wheel to monitor the circadian rhythmicity. However, running wheel is suitable for mice or hamsters, but not for rats, according to their spontaneous moving instincts (20).

The photocells/infrared beams for detecting

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the circadian rhythmicity are initiated in the studies of fruit flies (3), and now are common useful to most animals (2, 21). Using the photocells/infrared beams to measure the locomotor activity, one of the advantages is easily used and comparable due to its prevalence and commonness. The other most important advantage of photocells/infrared beams to the chronobiologists is still reliable during lights-off conditions, and the running wheel is another favorite tool because it has the same benefit as the photocells/infrared beams (2). At the same time, the photocells/infrared beams tools are more expensive (at least in Taiwan), and only one single animal enable to monitor by a set of apparatus.

Video tracking systems were introduced in the early 1990s, offering clear advantages of flexibility, spatial precision, and accuracy over the various hardware devices, such as the infrared beams and ultrasonic methods (15). A number of modern video tracking systems use frame grabbers to digitize analog video signals, and enable tracking of animals that are moving relative quick with high speed data acquisition. Moreover, the video tracking systems can be used to track couples of objects in one arena and continuously record the behaviors up to several days depending on the space of hard driver and sampling rate of computer.

Diurnal rhythm means that the patterns of activity or behavior follow the day-night cycles or environmental cues. Circadian rhythm is an endogenously generated rhythm with a period close to 24 h, the time of earth rotation. Specific criterion for circadian rhythm is that it must continue under constant conditions (*i.e.*, with no environmental cues, such as light) with a period still close to 24 h. Additionally circadian rhythm must be able to be phase reset by environmental cues. Therefore, basing on these advantages as above, we tried to validate whether the video tracking system was able to use for the chronobiologic research particularly to the rat's locomotor activity by its instinct. During the lights-on condition, the video tracking system successfully monitored and analyzed the locomotion of animals without any accessory has been reported. Using the infrared illuminators and a night-vision charge-coupled device (CCD) camera as the accessory instruments, the distance of rats' movement under non-visible light condition or under light-dark switch condition still can be recorded and analyzed in this study. Moreover, the phase-shifting event during constant darkness by the light-pulse interruption also exhibited. These results in the present study indicate that the video tracking system provides reliable data for studying the long-term diurnal or circadian rhythmicity in rats.

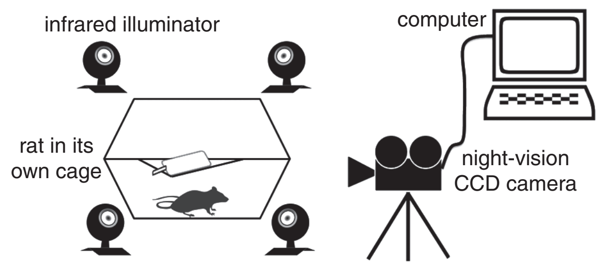

Fig. 1. Apparatus and equipment configuration used for the video tracking system in rats.

Materials and Methods

Animals

According to the power analysis (power = 0.8, confidence interval = 95%; deviation within 0.5), the minimal number of animals for each experimental design was 10. Therefore, 24 adult male Sprague-Dawley rats (250-280 g; National Laboratory Animal Center, Taipei, Taiwan) were used in this study. All animals were individually housed per cage in a temperature (22 ± 1°C)- and light-controlled room with free access to rat chow and tap water. For easily comparing the shifting effects of locomotor activity in the lights-entrained study, we used the Zeitgeber time (ZT), which is identified as the lights on, as the time index. Time of light-on in animal room was from 1900 to 0700 in this study. Each rat was kept in the standard rat polypropylene cage (45 cm in length, 30 cm in width and 30 cm in height) with corncob bedding to reduce the interruption of high frequency for changing the bedding (18). For smoothing the analysis of image in each rat, the rat cage did not provide any other housing item except the bedding. After one week of habituation and changing the bedding, rats were kept in non-visible light and infrared illuminators existed environment except the lights-entrained treatment (Fig. 1). All experimental protocols and procedures in the present study were approved by the Institutional Animal Care and Use Committee in Tzu Chi University. The institutional guidelines were followed for the care and use of animals and conducted in accordance with the European Community Council Directive of 24 November 1986 (86/609/EEC).

Principle of a Video Tracking System

Distance and duration of movements were measured and analyzed by the video tracking software (EthoVision 3.1, Noldus Information Technology, Wageningen, Netherlands). Beside the Etho Vision software, the whole video tracking system was equipped with a digital infrared sensitive CCD camera (A41I11N, Kera Electronics, Taipei, Taiwan), infrared illuminators (KE-101, Kera Electronics, Taipei, Taiwan), and personal computer. Locomotor activity of each individual rat in its own home cage was recording and six rats were used at the same time in each experimental process of this study. The first step is to detect the shape of each rat by the EthoVision software. Software calculated the mass center of each rat firstly, and then positioned by the coordinates of two axis (X_1,Y_1). Finally, the EthoVision software automatically calculated the distances of movement from (X_1,Y_1) to (X_2,Y_2) for each rat as the previous study (24).

Experimental Designs

During the entire procedures, animals were not disrupted with free access to rat chow and tap water. Rats were kept in 12:12 light-dark cycle for one week habituation before analysis of the video tracking system. In the first experiment, 12 male rats were kept in 12:12 light-dark cycle for two days and then lights were turned off but under infrared illuminators condition (<0.1 Lux) for the other six days. In the second experiment, 12 male rats were kept in the constant darkness condition for two days, and then exposed for one hour in 500 Lux white light condition to induce phase-shifting.

Statistical Analysis

The data were presented as mean ± standard error of the mean (SEM). One-way analysis of variance (ANOVA) with repeated measurements was used to test the significance of differences among time points. A value of $P < 0.05$ is considered statistically significant. Circadian rhythmicity was determined by cross-sectional analysis using the Cosinor procedure which assuming a 24 h period as described previously (14) with the freely available online program[1] as described previously (25); therefore, the ultradian rhythmicity was ignored by the program and not the main focus in this study.

Results

The apparatuses were briefly shown in the Fig. 1. The total distances of movements in rats were around 31000 ~ 34000 cm per day, and the average was near 32400 cm per day. Either in the light-dark conditions or in the constant darkness as well as in the short duration of light exposure condition in the present study, the average of moving distances in rats each day was about 32400 cm. Using this average (225 cm per 10 min) as the threshold of locomotor activity for determining pattern, the initiation of the locomotor activities was found around ZT 12:00-12:10 in the light-dark condition (Fig. 2A and 2B). Transforming the real moving distance (Fig. 2A, 2C, 2E, 2G, 2I, 2K, 2M and 2O) as the pattern activity (Fig. 2B, 2D, 2F, 2H, 2J, 2L, 2N and 2P), the pattern of locomotor activity showed as cluster pattern and was easier to distinguish as the rhythmic switch. Furthermore we used the shadow as the index of higher locomotion and the open as the index of lower locomotion as well as circadian patterns were also shown (Fig. 2).

The distance of movements under the light-dark condition exhibited the circadian rhythm ($P < 0.01$; Fig. 2A and 2B). While the rats were kept in constant dark environment, the distance of movements still exhibited the circadian rhythmicity ($P < 0.01$; Fig. 2C and 2D). However, the initiation time of the locomotor activities was subtle delayed for 10 ~ 20 min in the first and second days under constant darkness conditions, and the initiation time was ZT 12:10-12:20 (Fig. 2E) and ZT 12:20-12:30 (Fig. 2G). This delayed effect was continuously exerted until the end of this experiment (Fig. 2I, 2K, 2M and 2O), but the delayed duration was increased and showed 20 ~ 30 min from the third to the seventh days under constant darkness conditions (Fig. 2I, 2K, 2M and 2O). The initiation time was ZT 12:40-12:50 in the third day (Fig. 2I), ZT 13:20-13:30 in the fourth day (Fig. 2K), ZT 13:50-14:00 in the fifth day (Fig. 2M), and ZT 14:20-14:30 in the sixth day (Fig. 2O) under constant darkness conditions. Similarly using the shadow as the index of higher locomotion, the circadian patterns of locomotion showed the delayed events in the first day of constant darkness (Fig. 2F) and also showed the augment of delayed effects from the third day to the sixth day of constant darkness (Fig. 2J, 2L, 2N and 2P).

For verifying the efficiency of video tracking system also available to the detection of entrainment, we used the one-hour exposure of white light under the constant darkness condition in the next experimental design. Rats were kept in the light-dark conditions for two days, and then kept in the constant darkness condition while using the video tracking system. At this moment the beginning of constant darkness condition was defined as the first day (Fig. 3A). Under the constant darkness conditions, the moving distance of rats exhibited the circadian patterns ($P < 0.001$; Fig. 3A, 3C and 3E) as the previous data ($P < 0.01$; Fig. 2E, 2G, 2I, 2K, 2M and 2O). On day four, rats were exposed at the 500 Lux

[1] Circadian Rhythm Laboratory. University of South Carolina. Available from: http://www.circadian.org

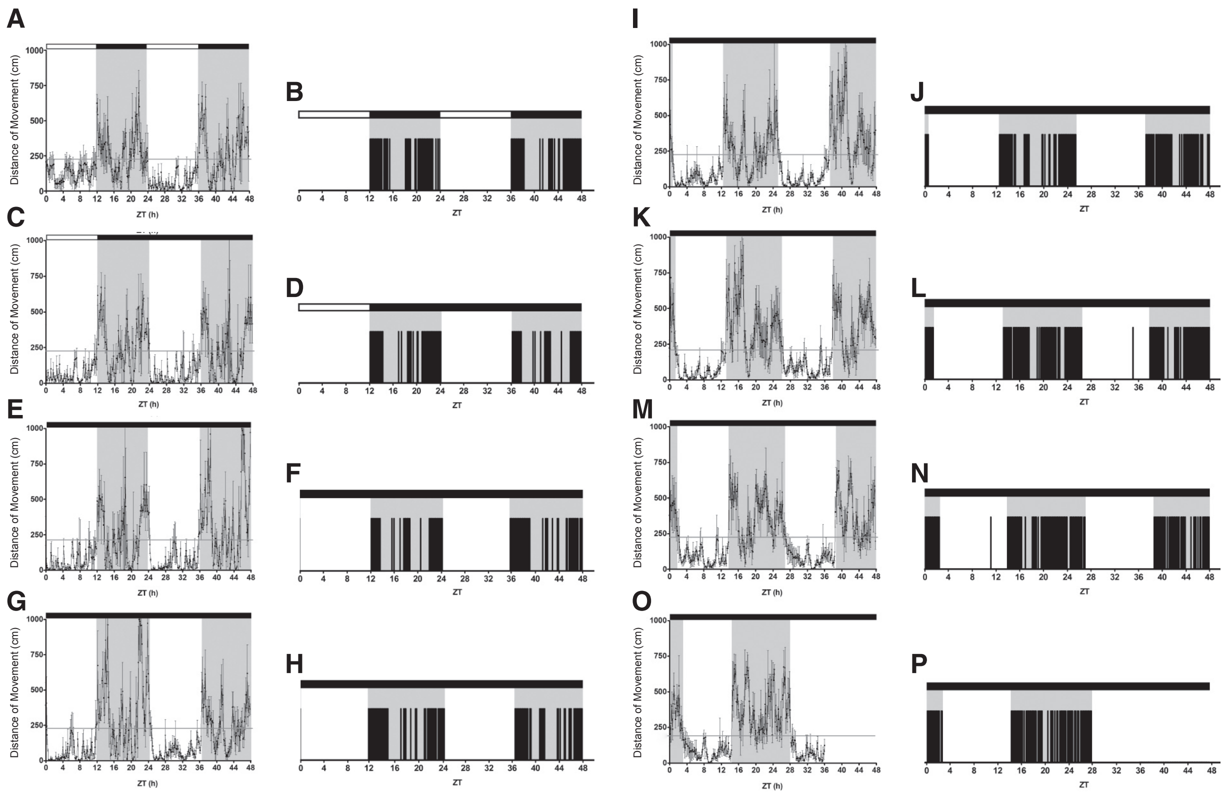

Fig. 2. Representative double-plot actograms of circadian rhythms in free-moving activity of rats under 12:12 light-dark cycle and constant darkness conditions. A, C, E, G, I, K, M and O panels represent the real moving distance of rats. Using the average of moving rates (225 cm per 10 min) as the threshold of locomotor activity (indicated by the horizontal light-gray line) for determining pattern and transforming the real moving distances (A, C, E, G, I, K, M and O) as the pattern activity, B, D, F, H, J, L, N and P panels represent the cluster pattern of locomotor activity. Open bars above the panels represent the light-on period and close bars above the panels represent the light-off one. Shadowed areas in the panels indicate the activity phase of locomotor rhythm. ZT indicates the light-on. The vertical lines of each dot at each time point represent the SEM (n = 12).

white light for one hour during ZT 20-21 (Fig. 3G). After this short period of light exposure, the rats were under the constant darkness conditions until the end of experiment. On next day after the light exposure (the fifth day), the circadian rhythmicity still existed in the following days ($P < 0.001$; Fig. 3I, 3K, 3M and 3O). However, the initiation time of the locomotor activities was changed comparing the periods before and after the white light exposure. Before the white light exposure, the initiation time of the locomotor activities was ZT 12:00-12:10 in the first day (Fig. 3A), ZT 12:00-12:10 in the second day (Fig. 3C), ZT 11:50-12:00 in the third day (Fig. 3E), and ZT 11:40-11:50 in the fourth day (Fig. 3G). On the other hand after the white light exposure, the initiation time of the locomotor activities was ZT 10:00-10:10 in the fifth day (Fig. 3I), and ZT 10:10-10:20 in the sixth (Fig. 3K), the seventh day (Fig. 3M), and the eighth day (Fig. 3O). The phase-shifting duration was advanced near 1.5-2.0 h. Similarly using the shadow as the index of higher locomotion, the phase-advanced effects by the white light exposure were shown (Fig. 3J, 3L, 3N and 3P).

Discussion

The results of the present study collectively demonstrate that the video tracking system was an effective and useful tool for the chronobiologic studies, at least in the circadian rhythmicity, under the non-disruptive conditions. The following findings provide this evidence. Locomotor activities in rats under the 12 h:12 h light-dark cycles showed the diurnal patterns (Fig. 2). The circadian rhythmicities in locomotion of rats under constant darkness conditions were also found (Figs. 2 and 3). Finally the entrainment and phase-advanced effects through the acute bright light exposure in rats' locomotor patterns were also validated (Fig. 3).

Due to the speedy development in hardware and software of computer sciences, the video tracking system was used more and more by behavioral sci-

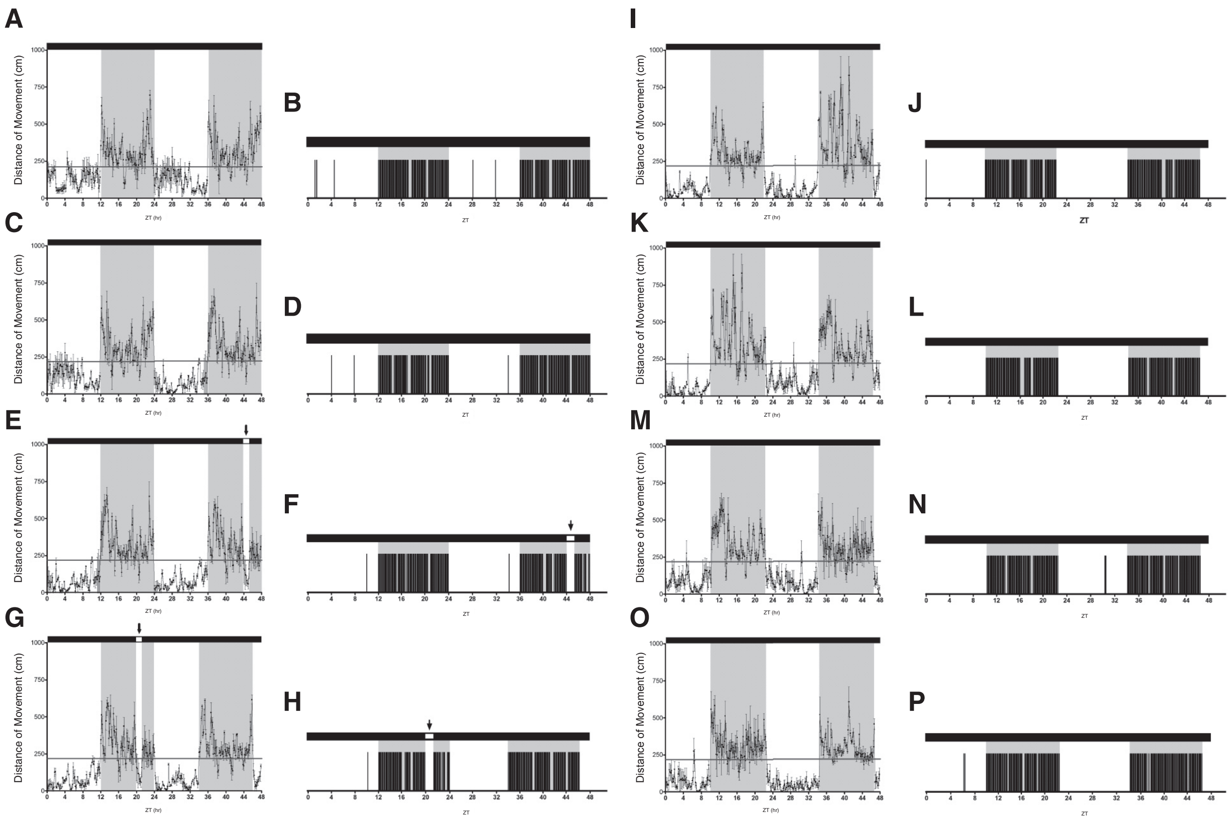

Fig. 3. Representative double-plot actograms of circadian rhythms in free-moving activity of rats under constant darkness with and without the light-pulse interruption conditions. A, C, E, G, I, K, M and O panels represent the real moving distance of rats. Using the average of moving rates (225 cm per 10 min) as the threshold of locomotor activity (indicated by the horizontal light-gray line) for determining pattern and transforming the real moving distances (A, C, E, G, I, K, M and O) as the pattern activity, B, D, F, H, J, L, N and P panels represent the cluster pattern of locomotor activity. Open bars above the panels represent the light-on period and close bars above the panels represent the light-off one. Arrows indicate the light-pulse interruption by one hour in 500 Lux white light. Shadowed areas in the panels indicate the activity phase of locomotor rhythm. ZT indicates the light-on. The vertical lines of each dot at each time point represent the SEM (n = 12).

entists. The video tracking system also has advantages of high spatial and temporal resolution, and these advantages let it become the major method for animal behavioral studies in the both laboratory and field environments (16). Ecologists and field behaviorists used the video tracking system to obtain the movement data which reflected the animal behaviors, to interpret the meaning of behaviors and even to predict the interactions with the environments (8, 10). These data from tracking system enable to be used to parameterize a wide range of ecological phenomena related to movement by scientists (9). In addition to the ecological or field studies, the video tracking system also has used for the laboratory animal studies, including the fruit fly (7), mosquito (23), zebrafish (22), and rodents (17). Although the video tracking system could be used for the detection of locomotor activity in the laboratory conditions, most scientists still used it for behavioral responses (5, 11, 17) rather than for circadian revelation (4, 19). Furthermore, even the video tracking system was applied to the rhythmic studies of vertebrates, and most studies just did the short-term (one or two days) application (4, 6, 13) but not the long-term (five or seven days more) one (19).

The common tool to detect the diurnal or circadian rhythmicities in rats and mice is the infrared sensor or photo beam detector. Its advantage is unrestricted by light density and light-dark transition, and one of its disadvantages is the cost. The cost of equipment by infrared sensor for each rodent is around US$1,000. On the other hand, the cost of video tracking system or software is also around US$1,000 ~ 2,000, but the video tracking systems can be used to track couples of objects in one arena. In the present study we used the video tracking system to simultaneously detect the locomotor activity in six rats. Therefore the best advantage of video tracking system is the cost-down effect, and even some laboratories are ready to provide the free or cheaper software.

However, the disadvantage of video tracking system is also existed. Most of limitations in video tracking system were to provide the indirect and uniform illumination under video-recoding conditions (16). If the light intensity was distributed unevenly, the video tracking system might identify the shadow as the tracking object. This disadvantage can be overcame by the experienced and standard operating procedure after the first success. Additionally another limitation of the video tracking system was the constant illumination conditions. Constant light-on or light-off (darkness) conditions were easy to avoid the error detections on the identification of tracking object. In the present study we successfully tracked the animals' locomotion under the light-dark and constant darkness conditions. Although we did not execute the constant lightness conditions in this study, because rats were nocturnal animals and rats kept in the constant lightness conditions was unhealthy and non-physiological. The final limitation of the video-tracking system was the capacity of hard drive (16), especially in the chronobiologic study. For example, the uncompressed video recording for 24 h might occupy more than 1.5 gigabyte (GB). For some special animal tracking conditions, the capacity of hard drive might need to be over 2 terabyte (TB) (16).

The other restriction in the present study was the rhythmic pattern of free running at constant darkness. According to most literatures, while rats were kept at constant darkness, the rhythmic pattern of free running should be early onset. In the present study, we found that this free running was delayed. The possibility or explanation is the bedding effect. Corncob bedding has been shown to contain no dust and reduce the spread of allergens (18). Corncob bedding also produces lower levels of ammonia within the cage, and thus minimizes the number of cage changes (18). However, the recent study also shows that corncob bedding decreased the slow wave sleep compared to aspen-chip bedding on rats by encephalography (12). Despite the acknowledged benefits of corncob bedding, rats sleep less on corncob bedding was found in the study (12) and this might also affect the rhythmic pattern of free running in the present study. Further studies to confirm the bedding effects are needed in the future, but this is not the main focus in this study.

In conclusion, using the night-vision CCD camera combined with the infrared illuminators, the video tracking system provides the reliable analysis for studying the long-term circadian rhythmicity in couples of rats at the same time.

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Conflict of Interests

The authors declare that they have no conflict of interests related to this work.

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