ヘアレスマウスの概日歩行活動および体温リズムの同調因子としてのブラックライト

Black Light as a Zeitgeber for the Circadian Locomotor Activity and Body Temperature Rhythms in Hairless Mice

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Light is reported to be the most important zeitgeber to entrain circadian rhythms in animals. However, effects of ultraviolet radiation (UVR) on the locomotor activity and body temperature rhythms have not been investigated well. Therefore, in this study, we examined the effect of UVA irradiation (black light) on the circadian rhythms of locomotor activity and body temperature in hairless mice. Female hairless mice (Hr-/Kud, 8 weeks old) were used as a material. Animal facilities were kept at 25±2 ℃ with 12 hour-light and 12 hour-dark cycle (LD 12:12). The amount of activity and body temperature were recorded by a temperature-sensitive telemeter system. Black light (UVA) entrained the locomotor activity and body temperature rhythms as well as white light. The amount of activity under the black light condition was significantly lower than that under the white light condition during light period. The ratio of locomotor activity during dark period to light period in the black light condition was significantly higher than that in the white light condition. These results suggest that UVA entrains the circadian locomotor activity and body temperature rhythms and tends to reduce the amount of activity.

Key Words: Ultraviolet radiation, Black light, Hairless mouse, Locomotor activity rhythm, Body temperature rhythm, Entrainment

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Introduction

Light-dark cycles have been considered to be the most reliable and important time cue (Zeitgeber) in mammals (Pittendrigh, 1981). Mice are able to sense ultraviolet radiation (UVR) while humans cannot see UVR. The reason is that mice do not have kynurenin filter which absorbs UVR. Diurnal animals such as humans, ground squirrels, monkeys and many fishes have kynurenin in the lens of the eyes and it absorbs UVR to protect the eyes from UVR (Gouras & Ekesten, 2004). In addition, it has another role to increase the resolution by reducing the scattering of light (Jacobs, 1992). However, it has not been known why mice need to sense UVR nor the physiological significance of sensing UVR.

Effects of ultraviolet radiation on the skin physiology and immunology have been studied well. We reported effects of UV irradiation on the histological changes in immune and endocrine organs of hairless mice (Sudo et al., 2006). However, other effects than the skin physiology and immunology have not been investigated so much. Visible light is reported to be the most important zeitgeber to entrain circadian rhythms in animals. However, effects of UVR on the locomotor activity and body temperature rhythms have not been investigated well. We examined the effects of solar simulated light with and without UVR on the locomotor activity and body temperature rhythms in hairless mice and found that both artificial solar radiation with and without UVR are a strong zeitgeber. (Chikamori et al., 2007). In this study, we examined the effect of UVR on the circadian rhythms of locomotor activity and body temperature by using black light, the spectrum of which is in UVA region, in hairless mice. We used hairless mice as a material to clarify the importance of the eyes and skin for the photoreception of UVR to entrain the circadian rhythms in future experiments.

Materials and Methods

Animals. As the material, 8 weeks old female hairless mice (Hr-/Kud, Kudo) with JcI:ICR background. The number of animals used was 8. These mice were divided into two groups and kept in large polypropylene cages (34×30×17 cm) for 7 days until the beginning of the experiment. During the experiment, the mice were placed individually in polypropylene cages (25×18×13 cm) and body temperature and locomotor activity were continuously recorded from 3 days after implantation of a telemeter in each mice.

All cages contained wood shavings (White flakes, Oriental Ferment Industries, JPN) with a steel mesh lid. They were fed rodent pellets (CE-2, Japan Clea Co, JPN). Water was available *ad libitum.* Change of wood shavings and water and replenishment of rodent pellets were done at the end of each experimental condition. Animal facilities were kept at 25±2 ℃ with 12 hour-light and 12 hour-dark cycle (LD 12:12; light on at 06:00 and light off at 18:00).

Light source. Mice were irradiated with white light (FLS40S/EX-N/MX 40W, Matsushita Co. Ltd.) in White light conditions without UVR. For UVA irradiation, they were subjected to irradiation with black light (FL40S.BLB type of 40W Black light, Panasonic). Animal facilities were kept with 12 hour-light and 12 hour-dark cycle (LD 12:12), and light was turned on at 06:00. The intensity of light was measured with a digital luminometer (T-10, Minolta) and a photon counter (S1137-66BQ, Hamamatsu Photonics). UV 340 (Sato Shoji) was used to measure the total amount of UVA and UVB, and MS-211-I (Eiko) was used to measure the amount of UVA.

The amount of UVA showed 0.5-7.0 W/m^2 and 29-312 kJ/m^2/12h .

Measurement of the amounts of activity and body temperature. The amounts of activity and body temperature were measured with temperature-sensitive telemeters (model XM-FH, Mini-Mitter Company, Inc. USA) calibrated with THERMO RECORDER (RY-10, ESPEC) in a water bath at temperatures around 30℃ and 40℃. Telemeters were implanted surgically intra-abdominally under ketamine-xylazine mixture anesthesia (Ketalar 50: 2% selactale: saline = 12: 3: 85) administered intra-abdominally. For awaking mice during surgery, we applied fluothane anesthesia. Data on the amount of locomotor activity and body temperature were collected at 5-min intervals using a personal computer (Aptiva, IBM) and software (Dataquest, Data Sciences International or Vital View, Mini-Mitter).

Experimental conditions. Experimental conditions were as follows. White light condition is from day 8-14, Black light condition is from day 15-24. Ambient temperature was kept at 27℃ constantly until the second day of Black light condition, but it was reduced to less than 25℃ because of failure of temperature control system.

Data and Statistical Analysis. The hourly value for each mouse in light and dark period were taken and then averages were calculated to get mean ±SD. Paired t-test was applied for comparisons between conditions. Differences were deemed statistically significant if $p<0.05$. χ^2 period analysis was done to find significant circadian rhythms. Differences were deemed statistically significant if $p<0.01$.

Results and Discussion

We obtained the data from 8 animals, but we used the results from 7 animals for analysis because of failure in the system in one of the mice. The results in locomotor activity and body temperature rhythms of White light condition were shown in Fig. 1. Both rhythms entrained clearly to the LD cycles and the period was close to 24 hours. Under Black light condition, both locomotor acivity and body temperature entrained to the LD cycles and the periods were 24 hours (Fig. 2). The amount of locomotor activity during light period is significantly lower than that during dark period both in white and black light conditions (Fig. 3). The amount of activity under Black light condition was significantly lower than that under White light condition during light period (Fig. 3). This result is in accordance with that in the previous report where artificially simulated solar light with UVR reduced the amount of activity in comparison with that without UVR (Chikamori et al., 2007). The ratio of locomotor activity during dark to light period in Black light condition was significantly higher than that in White light condition. The average body temperature during light period is significantly lower than that during dark period in both white and black light conditions (Fig. 4). These results suggest that (1) UVA is a strong zeitgeber for locomotor activity and body temperature rhythms, and (2) UVA tends to reduce the amount of activity and increase the ratio of the activity in dark to light periods. It would be interesting to clarify whether a specific UV photoreceptor pigment or a circadian photoreceptor (melanopsin) is involved in these UVR-induced phenomena in mice.

The average amounts (per 1 hour) of locomotor activity showed statistically significant differences in White light than in Black light condition ($p<0.05$, paired t-test or Wilcoxons test). The ratio between dark and light periods was significantly greater in Black light than in White

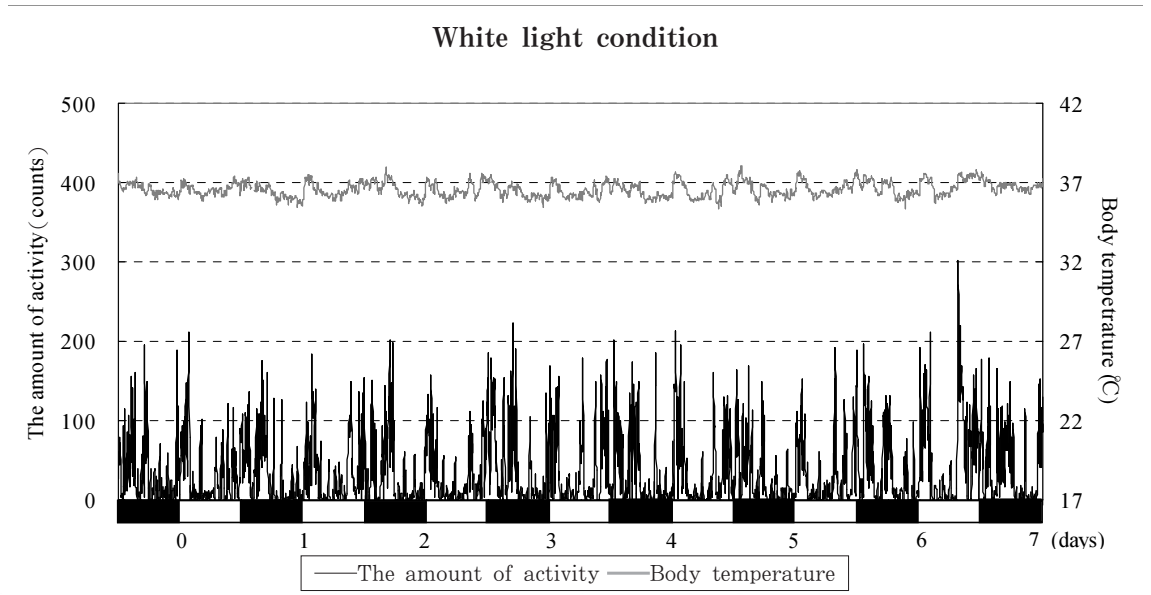

Fig. 1. Locomotor activity and body temperature rhythms of a hairless mouse under White light condition. Both the amount of locomotor activity and body temperature were low in light and high in darkness, and the rhythm was entrained to LD cycles.

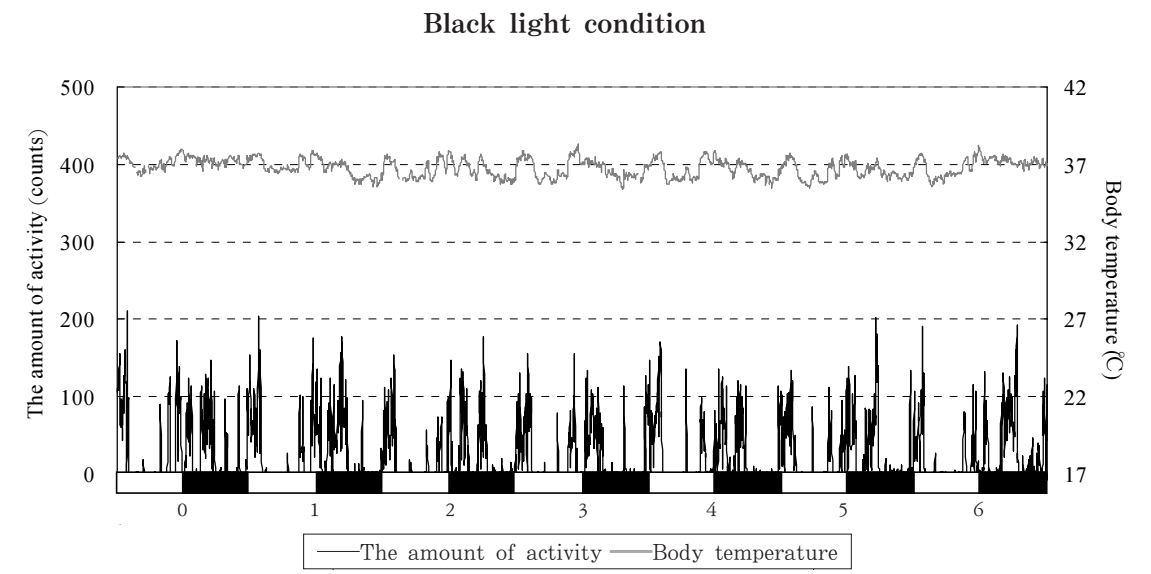

Fig. 2. Locomotor activity and body temperature rhythms of a hairless mouse under Black light (UVA) condition. Both the amount of locomotor activity and body temperature were low in light and high in darkness, and the rhythm was entrained to LD cycles.

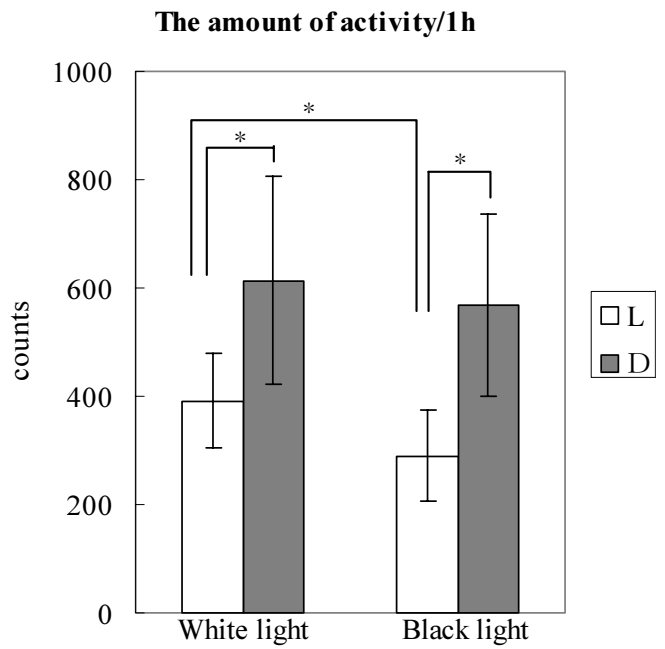

Fig. 3. Average amounts of locomotor activity per hour in light and darkness were shown. The amount of locomotor activity was significantly lower during light than during darkness in both White and Dark light conditions. The amount locomotor activity during light in Black light condition was significantly lower than that in White light condition.

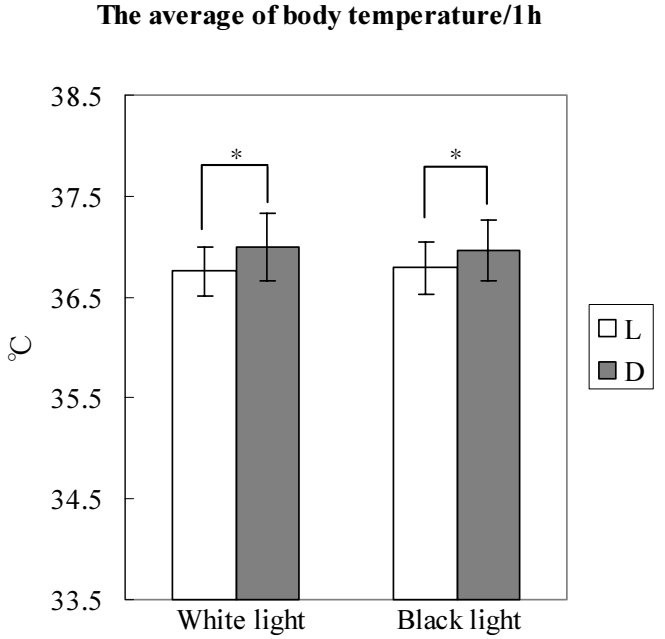

Fig. 4. Average body temperatures in light and darkness were shown. The body temperature was significantly lower during light than during darkness in both White and Dark light conditions.

light conditions (p <0.05, paired t-test).
The average body temperature per hour showed significant differences between light and dark periods in all groups (p <0.05, paird t-test or Wilcoxon test).

The above results have shown that the mice entrained to black light as well as white light under a LD 12:12 condition. In the previous report (Chikamori et al., 2007), we found that the locomotor activity was entrained to LD cycle of both UV- (VITA-LITE covered with Acryplen irradiation) and UV＋ (VITA-LITE irradiation) groups. The amount of activity was significantly greater in the dark than in the light period of LD cycles in both UV+ and UV- groups. There was no difference in the body temperature in the VITA-LITE group (UV+), but a significant difference was observed in the Acryplen group (UV-). These results suggest that the artificial solar radiation with UVR and without UVR is a strong zeitgeber for locomotor activity and body temperature rhythms. Therefore, the mice are able to respond to UVR as well as visible light, indicating the S cone is responsible for this response. However, since the black light contains small amounts of visible light, the response might have been due to the visible light. Bullough et al. (2005) reported that a light pulse (350 nm, 3mW/cm^2)put in the constant condition in mice (C57BL/6J, males) induced phase delay. These results strongly suggest that UVR is a Zeitgeber to entrain the circadian locomotor and body temperature rhythms in mice. The locomotor activity of European ground squirrels is not entrained to UVR, but it did respond to the ratio of log(310nm)/log(520nm) under natural condition only with the change of the ratio (Hut et al., 2000). A possibility that the nocturnal animal such as mice might also respond to ratio of log(310nm)/log(520nm) cannot be ruled out. Therefore, further experiments is necessary to clarify whether the mice have shown entrainment to UVA.

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ヘアレスマウスの概日歩行活動および体温リズムの同調因子としてのブラックライト

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可視光は動物の概日リズムを同調させる最も重要な因子として知られている．しかしながら，歩行活動と体温リズムに対する紫外線の影響については，ほとんど研究されていない．そこで，本研究においては，紫外線（ブラックライト）がヘアレスマウスの歩行活動と体温の概日リズムに及ぼす影響について調べた．

メスのヘアレスマウス（Hr-/Kud，8週令）を材料として用いた．動物飼育室は，温度条件は，25±2 ℃に保ち，光条件は，12時間明：12時間暗（LD 12:12）に設定した．UVAの光源として，ブラックライト(FL40S.BLB 40W, Matsushita)を用いた．歩行活動リズムと体温リズムは，温度感受性テレメーターシステム(Mini-Mitter）を用いた．概日リズムの周期の長さは，χ^2 ペリオドグラムにより解析した．

ブラックライト（UVA）は，白色光と同様，歩行活動および体温リズムを同調させた．ブラックライト条件における活動量は，白色光条件における値より有意に低かった．また，ブラックライト条件における明期と暗期の歩行活動の比は，白色光条件に比べて有意に高かった．これらの結果は，ブラックライトによるUVAがヘアレスマウスの概日歩行活動及び体温リズムの同調に関与するとともに活動量にも影響することを示す．

キーワード：紫外線，ブラックライト，ヘアレスマウス，歩行活動リズム，体温リズム