Strain Differences in Drinking and Eating Activities of the Inbred Mice

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STRAIN DIFFERENCES IN DRINKING AND EATING ACTIVITIES OF THE INBRED MICE[1,2]

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The present study was conducted to compare the rhythm patterns of drinking and eating among five strains of inbred mice (KR, CS, C57BL/6J, Mom, and KR-C/C). This was tested in the usual homecage after 3-days' habituation for 7 days. With both drinking and eating, all the strains showed the remarkable nocturnal rhythms which had two peaks, one immediately before sunrise and the other shortly after sunset, with a period of comparatively higher level of activity during night than during day. Both drinking and eating rhythms showed the close resemblance to each other in almost all strains. To make the strain differences clear, the strains were classified on the basis of the activity level (completely inactive vs. less active) and the duration of daytime phase [D] which showed the relatively stable and low level of activity (long vs. brief). Such a classification was confirmed as independent both of external and of internal variables. Mom showed the long and completely inactive [D] phase; [D] phase in C57BL/6J was long, but did not show a complete suppression of activity; CS mice had the brief and incomplete suppression; KR and KR-C/C showed the brief but completely inactive phase. Since the two strains of KR and KR-C/C which differed only in the C-c allele were put in the same group, the effect of albinism could be denied. The findings showed the high correspondence with the classification made on the basis of wheel-running in our previous experiment. As an additional test, two strains with mutant genes *ob* (C57BL/6J-ob) and *db* (C57BL/KsJ-db) were compared with the normal C57BL/6J mice. Polyphagia and polydipsia did not affect the pattern of activity.

Many investigations on the behavior genetics have been concerned with the differences in specific types of behavior among different strains of inbred mice (Dewsbury, 1978; Tsuji & Ebihara, 1980). Few of them, however, have conducted the comparison

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of different types of spontaneous behavior, in spite that the attempt would be a necessary step to determining the behavior trait of a strain.

The authors previously compared the nocturnal rhythms of wheel-running activity among twelve strains (eleven inbreds and one wild line) and tentatively classified them into three main types on the basis of the rhythm patterns (Ebihara & Tsuji, 1976). Such differences in the rhythm type proved to be independent both of the individual variables (age, sex, oestrus cycle, or partrition experience) and of the environmental ones (temperature, humidity, daytime, etc.). In the subsequent experiments, we compared the rhythm types between reciprocal F_1s to check the maternal effects and confirmed the determination of the types on the basis of the gene constitution.

However, it remains an open question whether such differences in the rhythm type were particular to the wheel-running or they indicate the regulatory function basicly influencing the various aspects of behavior in mice. The present study was designed to examine the above-mentioned point. It examined the within-day rhythms of drinking and eating and compared their types with those of the wheel-running activity.

Method

Animals. Seven strains of mice were used in the present experiment. Four of them, KR, CS, C57BL/6J, and Mom, were selected as the representatives from the different rhythm types in wheel-running. KR had the genetic constitution of AABBcc DDSS and was at over 70 generations of inbreeding. CS had its origin in the hybrids between NBC (N-group) and SII (hybrids of several groups) and its gene constitution was aabbccDDss. C57BL/6J, well-known strain (aaBBCCDDSS), was over 80 generations of inbreeding. Mom (A^wA^wBBCCDDSS), a Japanese wild mouse (*Mus musculus molossinus*), was under the process of inbreeding (from 8 to 10 generations) when the experiment was conducted.

KR-C/C (AABBCCDDSS), a congenic strain of KR, was used to test the effect of C-c allele upon the rhythm types.

The other two strains, C57BL/6J-ob and C57BL/KsJ-db, were added to examine the effects of major genes *ob* and *db* which might influence eating and drinking respectively.

ob (obese) is a recessive mutant gene and is located on the chromosome linkage XI (Ingalls, 1950). It has been found that the gene causes the polyphagia, obesity, hyperglycemia, the enlargement of Langerhans' islands, dysinsulinism (Mayer, 1960; Treble & Mayer, 1963). *db* (diabetes), on the other hand, is a single recessive gene belonging to the linkage VIII, and is reported to lead to the polyuria, the high degree of urine sugar and hyperglycemia, the degeneration of beta cells in Langerhans' islands and coma (Hummel, et al., 1965; Coleman & Hummel, 1967; Nishimura, 1976). The number of mice in these two strains was so limited that the data were just supplementary in the present study.

Apparatus Drinking and eating were recorded individually in the homecage of

aclylite, measuring 245(L)×175(W)×125(H)mm. The metal plates were placed under the food basket and the drinking bottle so that it could close the circuit when the mouse touched the basket of the bottle while standing on the plates. The circuit operated a corresponding channel of the pen-recorder. The cage floor was covered with shavings. Cages were placed side by side on the shelves of the animal room. Twelve mice from different strains were tested for the same period.

Measurement was made in the animal laboratory at the Department of Psychology, Nagoya University. Temperature was controlled at the constant level of 23.0±0.5°C, and humidity at 60±5%. Lighting was not controlled; natural sunlight was supplied through the window[6]. Thus the light intensity changed gradually from light to dark and vice versa. Average level of lighting was approximately 300 lux at the top of homecage.

Procedure Mice of the five strains (KR, CS, C57BL/6J, Mom, KR-C/C) were tested at their ages of 30, 40, 50, 60, 90, 120, 150, and 180 days. Mice were housed individually and their activities were recorded for ten days, from five days before through four days after the prescribed age. Records for the first three days, however, were discarded in the analysis, since the mice might not have become habituated to the test situation during initial period. Thus the records for the last seven days were used for analysis.

Results

Rhythms of drinking and eating activities Rhythms of both activities were obtained by averaging the samples of seven days for every hour of the day. This procedure, however, presupposed the change with a cycle of 24 hours. To test its validity, one male mouse was sampled from each of the five strains and its drinking and eating were plotted from day to day. All the mice showed remarkable nocturnal rhythms with a cycle of 24 hours. When the mean score of seven days was taken, the effect of artifact, even if it had occurred, would have been cut down to one-seventh and the effect of oestrus cycle in the adult females would have been made negligible. We also examined how long the mouse contacted an empty basket or bottle in order to obtain the measure of activity not relating to the food or water consumption. As the result, we succeeded in making it clear that the rhythms showed the actual drinking and eating.

General characteristics in drinking and eating activities Rhythms were obtained with the sex and age of mice combined for our analysis of general characteristics (Figures 1 and 2). Drinking and eating rhythms resembled each other, which was demonstrated by the high correlations between these two measures taken from same hours of the same day (Figure 3). Both activities showed remarkable nocturnal rhythms in all of the five strains. The activity in night-time, however, was not kept at a constant level; two

6. To increase the light intensity in the room, fluorescent lamps were lit during daytime by an operation of the light sensor set outdoors.

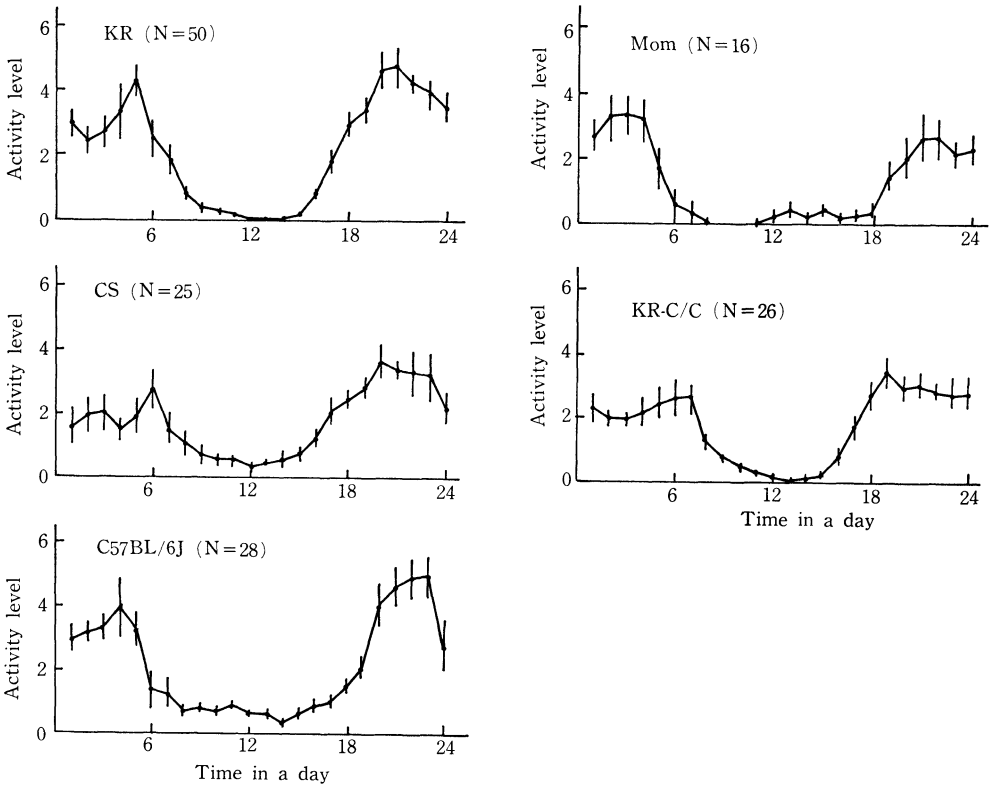

Fig. 1. Rhythms of drinking activity obtained from five strains. Data from males and females were combined. Vertical lines denote the range of 1 SD.

peaks appeared at the time of sunrise and just after sunset, and the activity level was comparatively low between them.

Strain differences in the rhythm types The characteristics of the rhythm in each strain were summarized as follows, with both activities taken into consideration.

KR. The first peak appeared at 5:00 and activity decreased thenafter till 8:00. The mouse became completely inactive during daytime and began to show gradual increase in activity from 16:00 to the second peak at 21:00. The bottom of activity appeared at 2:00.

CS. The activity came to the first peak at 6:00 and then leveled down until 8:00. It was kept at a low level during daytime and began to increase from 16:00 to show the second peak at 20:00. The rhythm of this strain was characterized by the smaller difference in level between night and day.

C57BL/6J. The first peak appeared at 4:00. Although the level in daytime was as high as that of CS, the bigger amount of activity in night-time made the rhythm of this strain more remarkable than that of CS. The second peak appeared rather flat and ranged from 20:00 to 23:00.

Mom. Level of activity was the lowest of all the strains used in the experiment,

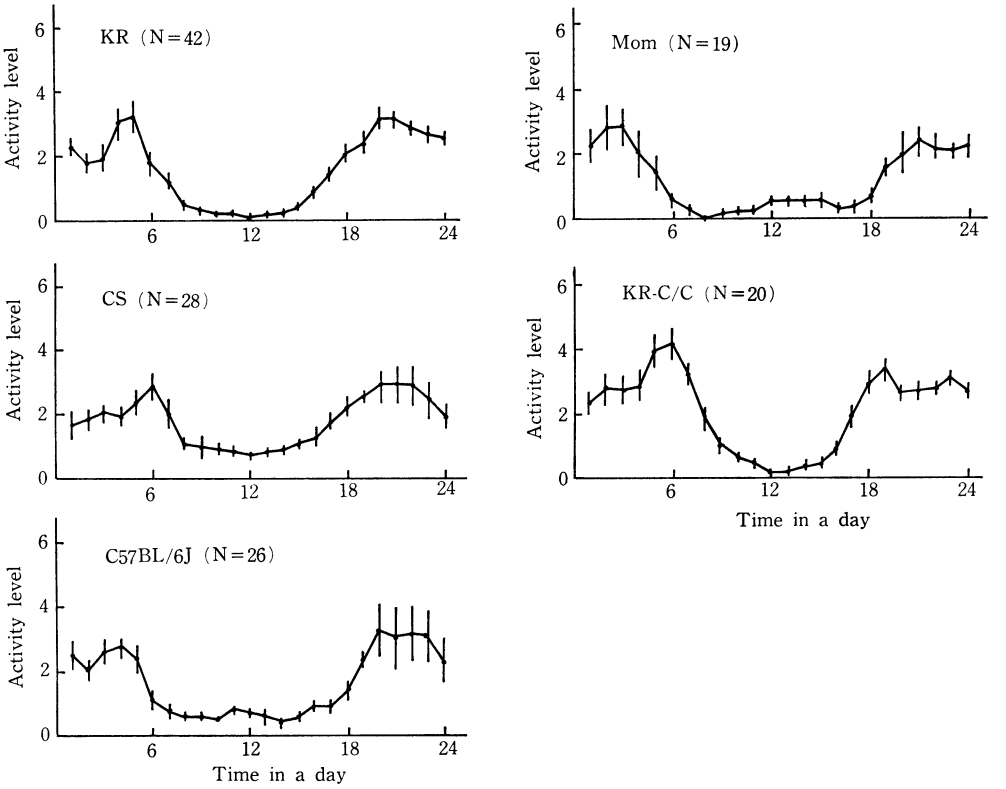

Fig. 2. Rhythms of eating activity obtained from five strains. Data from males and females were combined. Vertical lines denote the range of 1 SD.

probably because its small body size needed less amount of food and water. Activity was completely inhibited in daytime and the active phase could be discriminated from the inactive phase most clearly, although the two peaks seemed somewhat indistinct.

KR-C/C. Activity pattern highly resembled that of KR, with an exception that the peaks were rather indistinct in this strain.

To make the strain comparison easier, the curves of each strain were segmented into the following four phases: [N], the phase in which the activity was relatively high between the second and the first peaks; [D], the phase which showed the stable and low level of activity; [ND], the transient phase in which the activity leveled down abruptly; [DN], the transient phase which showed abrupt increase in activity. The time range and the duration of these phases are shown in Table 1. As for [N], its duration, the level, and the position of bottom could be compared among strains. Data, however, showed too wide deviation to make any concluding results. Strain comparison, therefore, was attempted by means of the duration and the level of [D]. The result is shown in Table 2. Since KR-C/C was classified in the same group as KR, the effect of albinism upon the rhythm types could be denied.

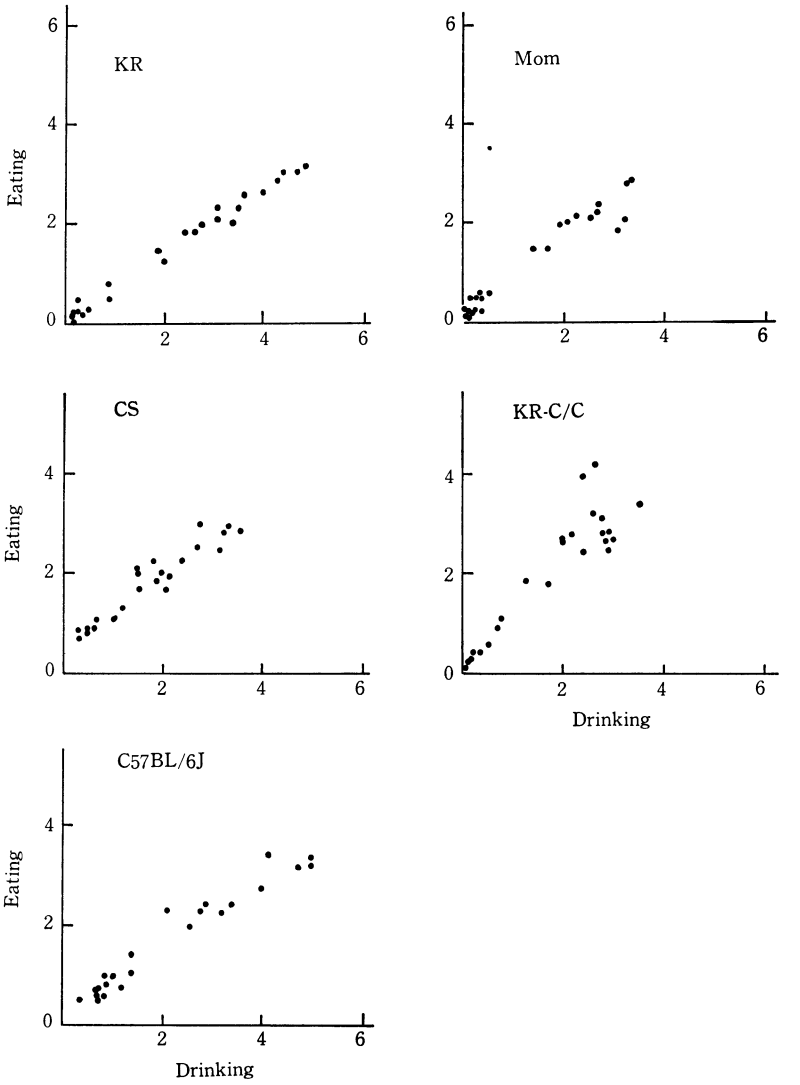

Fig. 3. Correlations of the activity levels between drinking and eating.

Examination of organismic and environmental variables upon the rhythmtypes We observed the strain differences in rhythm by combining the age and the sex of mice obtained at the different time of year. Then, the data were rearranged to examine whether organismic and environmental variables produced any effect in determining the rhythm types. The results were as follows.

Sex. No sexual differences were found out. The female's oestrus cycle did not affect the rhythm types.

Age. The duration and the level of [D] did not change with the age of days.

Season. Since the test was conducted throughout the year, the time of sunrise and

Table 1. Comparisons of the time range and the duration of each phase in the rhythm among five strains.

	Strain	[N]	[ND]	[D]	[DN]
Drinking	KR	21-5 9	6-8 3	9-15 7	16-20 5
	CS	21-6 10	7-8 2	9-15 7	16-20 5
	C57BL/6J	21-4 8	5-6 2	7-17 11	18-20 3
	Mom	22-4 7	5-6 2	7-18 12	19-21 3
	KR-C/C	20-7 12	8-9 2	10-15 6	16-19 4
Eating	KR	21-5 9	6-8 3	9-15 7	16-20 5
	CS	21-6 10	7-8 2	9-15 7	16-20 5
	C57BL/6J	21-4 8	5-6 2	7-17 11	18-20 3
	Mom	22-3 6	4-6 3	7-18 12	19-21 3
	KR-C/C	20-6 11	7-9 3	10-15 6	16-19 4

Upper values indicate the time range and lower values indicate the duration of each phase.

Table 2. Types of the five strains classified by drinking and eating rhythms

Level	Duration	
	long	brief
low	C57BL/6J	CS
zero	Mom	KR KR-C/C

sunset changed from season to season, which might have affected the rhythm types. If the rythm had been exogenously determined, it would have changed correspondingly with seasons; i.e., the duration of [D] was expected to be longer in summer than in winter. Although slight differences were observed among seasons, it did not show any correspondence with the expected rhythm types as mentioned above.

Effects of the single mutant gene upon the rhythm types The rhythms observed with C57BL/KsJ-db (hereafter referred to as *db* for short) and C57BL/6J-ob (referred to as *ob*) are shown in Figures 4 and 5 respectively. The *db* mice showed the higher level of drinking and eating during night, but their nocturnal rhythm was made less remarkable than the normal C57BL/6J mice, since their activities were kept at comparatively high level even in the daytime. The *ob* mice showed the remarkable drinking rhythm

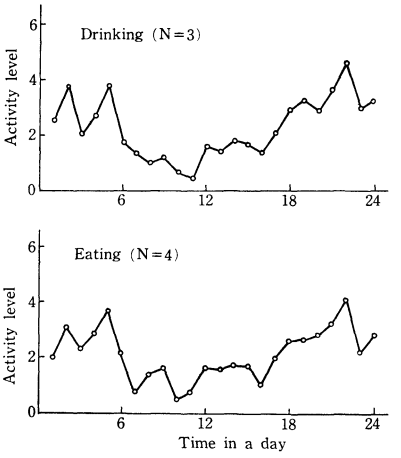

Fig. 4. Rhythms of drinking and eating in C57BL/KsJ-db mice.

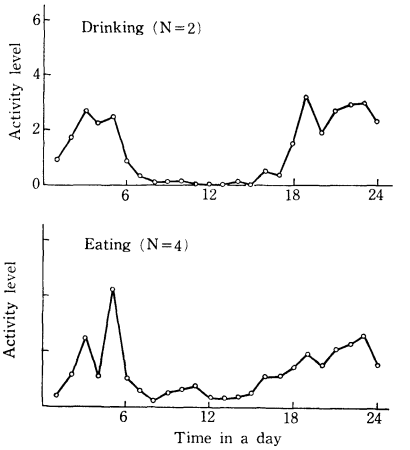

Fig. 5. Rhythms of drinking and eating in C57BL/6J-ob mice.

in which the activity during daytime disappeared more completely than the C57BL/6J mice, while the eating rhythm was rather blurred because of less amount of activity in night-time.

It was noted that there seemed to be no difference in the amount of activity between these mutant strains and the normal C57BL/6J. In spite of the fact that the amount of food and water consumed by *db* and *ob* strains was greater than the normals, the scores were at the similar level for both of them. This suggests that the rate of drinking and eating must have been greater in these mutant mice. The effects of environmental and organismic variables were not examined because of the smallness of the sample size.

Discussion

In the present experiment the following findings were obtained. 1) With both drinking and eating, the remarkable nocturnal rhythms were shown for all the strains; i.e., the rhythms had two peaks, one at the time of sunrise and the other immediately after sunset, with a period of comparatively higher level of activity during night than in daytime. 2) The drinking rhythm showed close resemblance to the eating rhythm in almost all the strains. 3) To make the strain differences clear, the strains were classified on the basis of the activity level (completely inactive vs. less active) and the duration (long vs. brief) of [D] in which the activity was relatively stable. 4) Such a a classification was confirmed as independent both of environmental and of organismic variables. 5) Two strains of KR and KR-C/C, which differed only in the C-c allele, were put in the same cell of our classification, although the close examination disclosed slight differences between them. 6) C57BL/6J-ob, the mutant strain with dysfunction of eating, was compared with C57BL/6J and was found to be of the same type with an exception of less amount of difference between night and day. Likewise,

C57BL/KsJ-db, the strain with dysfunction of drinking, showed the similar type of eating rhythm to C57BL/6J, while drinking completely disappeared during daytime in this strain.

Some researchers were concerned with the interaction between drinking and eating activities. Reporting that the matured rats showed the drinking activity of 2–3 hours' period, Richter (1926) denied the interaction between these two. Strominger (1947) emphasized the close relation between the two by demonstrating that the ratio of the daily consumption of food and water was kept constant, and that the amount of the consumed food decreased with restriction of the water supply in rats. As for the activity rhythm, Siegel & Stucky (1947) measured the amount of consumed food and water with rats four times a day (6:00, 12:00, 18:00, 24:00) for three days, and found that both food and water were consumed more from 18:00 till 24:00 and least from 12:00 till 18:00, which suggested the close relation between them.

Kutscher (1974) compared the amount of water consumption under three conditions, ad lib feeding, restricted feeding of 1/3, and food deprivation, among seven strains of inbred mice (SWR/J, C3H/HeJ, CBA/J, DBA/2J, BALB/cJ, A/J, C57BL/6J). In all the strains, positive correlations were obtained between the body weight and the amount of consumed food and water respectively.

In the present experiment, too, the high correlation was found between drinking and eating. Moreover, such high correlation was confirmed as independent of the other variables. In view of the activity rhythm, these two kinds of activity should be considered as indicating the general regulatory function.

The authors previously examined the rhythm types of wheel-running in twelve strains of mice, as was described earlier. We proceeded to compare both types of rhythm. We reclassified the corresponding strains so as to fit the grouping of the present study, since our former classification was based only on the activity level during daytime. The result is shown in Table 3. Both classifications showed the high correspondence, although some differences were observed in the details of data.

Table 3. Types of the five strains classified by wheel-running rhythm

Level	Duration	
	long	brief
low	C57BL/6J	CS
zero	Mom	KR KR-C/C

In the wheel-running activity the Mom mice were classified as Type A′ to discriminate from Type A because of the slow habituation to the apparatus and the longer duration of running after habituation. The present experiment, however, was conducted in the homecage and the mice had been allowed three-days' period to habituate, which may have reduced the possible activation of fear aroused from the novelty of situation.

Actually, the mice showed the suppression of drinking and eating on the same day when they were moved into a new cage. In spite of the difference seen in the habituation process, the types of drinking and eating activities were similar to that of wheel-running.

Another discrepancy was found in the rhythm of CS mice. CS mice showed rather distinct nocturnal rhythm in drinking and eating, while their wheel-running rhythm was extremely blurred. We may mention the following four as the possible factors which may contribute to the discrepancy.

In the first place, the scoring methods were different from each other. The scores 1, 2, 3, 4 corresponded to 1-2 rph, 11rph-10 minutes' duration, 10-30 minutes' durtion, over 30 minutes' duration respectively in our previous experiment. Such a scoring procedure may have deformed the peaks which would normally appear immediately before sunrise and shortly after sunset. In the present study, five-minutes' duration of drinking and one-minute's duration of eating were scored as one respectively, and thus the scores approximately corresponded to the real duration of activity. However, CS in the present experiment showed the greater difference in level between night and day. This result could not be explained by this factor alone.

Secondly, the difference in environmental variables might be mentioned. In the former experiment mice were maintained in the room where the higher level of noise than that in the present experiment was generated from inside and outside. In our ordinary observation, CS mice seemed to react to the sound by activating themselves. Such a difference in the noise intensity may have affected the amount of activity during daytime and may have produced the discrepancy of the rhythm types.

Thirdly, in the former experiment, the mice were fed once a day in the morning during the test session. Yagi (1961) reported that the rat's wheel-running activity was conditioned to the feeding time with the increased activity immediately before feeding. Closely examined, CS showed a small, vague peak centered at 9:00. This may be due to such an effect. Of course, the feeding schedule was the same for all the strains, but the CS mice were considered to be most sensitive to the artifact, as was seemingly consistent with the tendency mentioned above.

Finally, the CS mice seemed to manifest individual differences in behavior more remarkably than the other inbred strains, although we have not accumulated enough evidences for that yet. The average curve disclosed the distinct nocturnal rhythm in the present experiment, but some of the mice showed the apparent lack of rhythm. In connection with the other types of behavior, we have to pursue the basic trait of this strain in the further study.

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