Coupling effect of locomotor activity on the rat’s circadian system

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Lax, Pedro, Salvador Zamora, and Juan A. Madrid. Coupling effect of locomotor activity on the rat’s circadian system. Am. J. Physiol. 275 (Regulatory Integrative Comp. Physiol. 44): R580–R587, 1998.—Exercise is recognized to affect circadian rhythmicity in a variety of ways. It masks the expression of other behavioral and physiological rhythms, entrains the master pacemaker, and influences the free-running period of other rhythms. In this paper we study the influence of exercise on the organization of the timing system by analyzing the effect of voluntary locomotor activity on the circadian feeding behavior of rats subjected to different lighting conditions. The availability of wheel running prevented loss of feeding circadian rhythmicity under constant bright light (LL) but did not elicit any circadian pattern in rats showing a previous arrhythmic pattern. Under dim red light (DR), the rhythm was more pronounced in exercising than in sedentary rats, while wheel-running availability accelerated the emergence of circadian rhythmicity in arrhythmic animals that were moved from LL to DR. These results can be explained by the existence of a positive feedback loop between physical exercise and its pacemaker and also suggest that exercise changes the functioning of the circadian system to facilitate the emergence of circadian rhythms in previously arrhythmic animals.

Circadian rhythms; feedback; wheel running; feeding behavior

In mammals, the circadian system is timed by one or more central pacemakers, which entrain to environmental cycles by resetting or phase shifting the endogenous rhythms in response to external stimuli. In the absence of environmental cues, central oscillator(s) free run with a period close to 24 h (1), so that pronounced circadian rhythms persist in constant darkness or dim red light (DR). However, long-term exposure to constant bright light (LL) reduces the coherence of rhythmicity and can result in the loss of rhythmicity within the circadian range (6, 7, 22).

Apart from light-dark cycles, which are the most important zeitgeber in mammals, modulated access to food (21) or to water (20), environmental temperature (2), social contacts (24), and periodic noise (17) have been recognized as influencing the circadian system. However, not only environmental cues influence such circadian timing. It is known, for example, that cycles of modulated locomotor activity can reset the circadian clock, and the circadian rhythms of animals kept under constant light or dark can be entrained by a scheduled daily exercise (9, 18). Moreover, there is evidence that behavioral activity modifies the rate of phase shifting (25), modulates the period of the central pacemaker(s) (11, 26), alters the phase of photic entrainment (19), and influences the expression of other rhythms such as sleep and wakefulness (29), drinking behavior (10), and feeding pattern (28). Shioiri et al. (26) demonstrated a positive correlation between motor activity and 5-HT concentration in the suprachiasmatic nuclei (SCN), which suggests that exercise affects the central control of rhythmicity.

As the circadian system becomes more fully understood, feedback effects are progressively emerging as important components in the circadian clock’s organization. They can be considered as regulatory loops in which outputs act on the pacemaker(s), modulating its activity. Phenomena such as splitting and the presence of certain irregularities at the onset and termination of the activity phase in free-running conditions cannot totally be explained without taking feedback loops into consideration (8). Circadian locomotor activity could provide feedback cues to the central pacemaker(s), thus modulating circadian rhythmicity. Although circadian feedback loops are an important element in the physiology of the circadian system, their precise role in the neurobiology of the circadian regulatory system is still not completely understood.

The circadian clock can be considered as a multioscillatory system composed of at least two distinct oscillators (8, 23). Under natural conditions, or under constant darkness, the timing system could function in a coupled fashion, generating stable circadian rhythms. However, prolonged exposure of the animal to LL could uncouple components of the multiple oscillator, resulting in the above-mentioned loss of rhythmicity and temporal disorganization of the system. The aim of the present study is to examine the feedback effect of unscheduled voluntary exercise on the circadian system during temporal disorganization produced by exposure to bright light and during the transition from an uncoupled to coupled state of the system and vice versa. For this, the feeding behavior was recorded in rats subjected to different schedules of wheel-running availability and lighting conditions.

MATERIALS AND METHODS

Animals and housing. A total of 44 male Sprague-Dawley rats was used in the four experiments carried out. Animals were individually housed in modified Perspex cages, which permitted feeding activity to be recorded. Some of the cages were provided with a running wheel in which animals were allowed to engage in unscheduled voluntary exercise. The cages were housed in quiet, temperature- and humidity-controlled rooms (23 ± 1°C and 60%, respectively) and exposed to different lighting conditions, according to the experimental procedure. A pelleted diet (Letica, Barcelona, Spain) and tap water were available ad libitum. Food was weighed and replaced twice a week at different times during the light period to prevent synchronization. The cages were
cleaned, and the animals' body weights and food intake were recorded weekly.

Apparatus. Modified rat-keeping cages provided with contact eatometers were used for feeding registration, as described in detail elsewhere (15, 16). Briefly, each transparent polycarbonate rat-keeping cage (Panlab) was covered by a stainless steel grid. Mounted on the inside of the feeding area was a hinged grid that, when pushed up, enabled the animal to reach the food. To prevent false contacts with the food hopper as a result of spontaneous motor activity of the rats, a U-shaped stainless steel protector was mounted on the bottom of the swinging grid. A microswitch fitted to the swinging grid was activated each time the grid was pushed. To stimulate the realization of physical exercise, a running wheel mounted in a transparent frame was placed in the cage. An eccentric cylinder, attached to one end of its axis, was coupled to a microswitch to record wheel revolutions. This information was then fed into an IBM PC-AT computer with an I/O (CIO-DIO-96, Computer Board) card, which received a signal from the sensors every time they were activated. Feeding behavior was monitored as long as the microswitch was activated (feeding duration), while wheel running was measured as wheel revolutions (15). All measurements were read online by the microcomputer and stored on a floppy disk every 10 min. Subsequent calculations were based on these 10-min counts.

Procedure. In experiment 1, rats \( n = 8 \) were kept under a 12:12-h light-dark cycle (LD) from birth to weaning. On day 21, the animals were divided into two separate groups \( n = 4 \) and placed under LL \( (300 \text{ lx}) \). Cages from the first group were provided with running wheels (WR + ), which allowed the animals to engage in unscheduled voluntary exercise. On day 100, the running wheels were removed from group 1 and placed in the cages of group 2. Feeding activity was recorded from weaning to 20 days after the running wheels were removed.

In experiment 2, rats \( n = 10 \) were kept under LD from birth to weaning (day 21), when the lighting conditions were changed to DR \( (\text{light intensity } < 0.25 \text{ lx}) \). On day 35, the animals were divided into two separate groups \( n = 5 \), and the cages from the first group were provided with running wheels. From day 49, all the animals were subjected to LL for 30 days. Feeding activity was recorded from day 49 to day 80.

In experiment 3, rats \( n = 10 \) were kept under LD conditions from birth to weaning (day 21), at which time LL was imposed. On day 35, the animals were divided into two separate groups \( n = 5 \), and the cages from the first group were provided with running wheels. Thirty days later, all the animals were subjected to DR for 40 days. Feeding activity was recorded from day 35 to day 105.

In experiment 4A, rats \( n = 8 \) were kept under LD from birth to day 10, at which time LL was imposed. At weaning (day 21), the animals were divided into two separate groups \( n = 4 \). The first group was allowed to wheel run from weaning, while group 2 was not allowed to realize any locomotor activity. In experiment 4B, the animals \( n = 8 \) were subjected to LL from birth. The animals were divided at weaning into two groups \( n = 4 \), one of which was allowed to wheel run. Feeding activity was recorded from weaning to day 70.

The four experimental procedures are summarized in Table 1.

Data treatment. The computerized device continuously recorded the feeding activity for every rat throughout the experiment. The beginning and end of each experimental day was considered as midday, which corresponds to the moment of least activity. Chi-square periodograms (27) and Fourier analysis (Cronobio-PC, V3.0, Panlab) were obtained by averaging 10-min data bins. In the case of records in which the periodogram was not significant, the best period of the free-running rhythm \( (\tau) \) was used for the Fourier analysis. The differences between groups in the mean amplitudes of the first harmonic were statistically analyzed by ANOVA. If there were significant effects, individual comparisons were made by means of Scheffé's test.

RESULTS

Experiment 1. From the beginning of records (day 21), LL, the rats from both groups \( (\text{WR} + \text{ and those without running wheels } (\text{WR} -)) \) showed different patterns of feeding behavior. Rats allowed to wheel run \( (\text{group 1}) \) displayed a circadian feeding rhythm, with a period ranging from 25 h 20 min to 26 h 10 min, whereas sedentary animals \( (\text{group 2}) \) exhibited no circadian rhythmicity \( (\text{Fig. 1}) \). When the running wheels were removed (day 100), the rats from group 1 showed a noticeable loss of rhythmicity, the resulting periodograms being significant only for one of the four animals. However, when running wheels were made available to the rats from group 2 (day 100), they did not acquire any circadian rhythmicity \( (\text{Fig. 1}) \). Fourier's analysis showed a higher amplitude for rats allowed to wheel run from weaning than for those not allowed to realize locomotor activity \( (F_{3,12} = 5.95, P = 0.01) \). When

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Experimental procedure used in the 4 experiments. Day indicates age of animals at beginning and at end of each experimental phase; \( n \) indicates number of animals used in each experimental group; Phase indicates lighting conditions and wheel-running availability in each experimental phase (LD, 12:12-h light-dark cycle; DR, dim red light; LL, constant bright light; WR+, wheel-running availability; WR−, wheel-running deprivation).
running wheels were removed, circadian feeding rhythmicity showed a weak, not statistically significant, diminution in the amplitude in rats from group 1 (Fig. 4).

Experiment 2. Recorded feeding activity under DR showed a circadian rhythmicity in all animals, the free-running periods ranging from 24 h 10 min to 24 h 30 min (Fig. 2). Exposure of the animals to LL (day 49) induced a progressive loss of circadian feeding rhythmicity in both groups (WR+ and WR−). However, this loss of rhythmicity was slower in rats allowed to wheel run than in rats without exercise. Thus periodograms realized for the first 20 days after the light was switched on showed significant rhythmicity in all five WR+ animals, whereas only one WR− animal out of five displayed any significant periodicity. Fourier’s analysis showed both a higher amplitude in the rhythms of WR+ than in WR− rats under DR (F3,16 = 17.4, P < 0.001) and a more gradual decrease in the amplitude of the rhythms in the former after LL imposition (Fig. 4).

Experiment 3. In this case, all animals (both WR+ and WR−) showed an absence of circadian feeding rhythmicity under LL from the beginning of the records. Fourteen days after LL imposition, none of the periodograms showed a significant profile. However, a few days after the lighting conditions were changed (LL to DR), all the animals acquired a free-running rhythm, although rhythmicity did not appear at the same time in all the rats, with exercised animals acquiring a circadian periodicity in feeding behavior earlier than sedentary animals. Thus periodograms made from 5 to 25 days after switching off the lights showed significant rhythmicity in all five WR+ rats, with periods ranging from 24 h 10 min to 24 h 20 min, whereas no rhythmicity appeared in any of the five WR− animals (Fig. 3). Fourier’s analysis showed an earlier and higher increase in the amplitude of the rhythms of WR+ than WR− rats (F1,8 = 6.62, P < 0.05) (Fig. 4).

Experiment 4. After weaning, all the animals kept under LL from birth showed robust circadian rhythms independently of running-wheel availability, with free-running periods ranging from 24 h 50 min to 25 h 40 min. However, this rhythmicity was not so clear in rats kept under LD from birth to day 10 and under LL from day 10 to the end of the experiment, with only one animal, corresponding to group WR+, showing firm and stable rhythmicity, whereas the rest of the animals
exhibited significant but unstable circadian patterns. Fourier's analysis showed differences in the amplitude of rhythm of animals from different groups ($F_{2,7} = 4.61$, $P < 0.05$). Thus animals kept under LL from birth showed the highest amplitude, whereas animals kept under LD from birth to day 10 and not allowed to wheel run exhibited the lowest. Animals kept under LD from birth to weaning and allowed to realize locomotor activity showed an intermediate amplitude in their circadian rhythmicity (Fig. 4).

Food intake. Despite the increase in energy expenditure produced by physical exercise, voluntary wheel running did not alter significantly food intake ($F_{3,16} = 2.49$, $P = 0.09$). The mean food intake between day 35 and day 49 varies from $25.1 \pm 2.65$ g/day in LL WR$-$ ($n = 5$, expt 3) to $21.82 \pm 2.36$ g/day in LL WR$+$ ($n = 5$, expt 3). Similar results were obtained in rats subjected to DR light, $25.02 \pm 2.94$ g/day in DR WR$-$ ($n = 5$, expt 2) vs. $21.40 \pm 2.07$ g/day in DR WR$+$ ($n = 5$, expt 2).

**DISCUSSION**

The effect of exercise on different aspects of circadian rhythmicity has been widely analyzed, and, to date, authors have found the following: 1) voluntary motor activity can influence the expression of other behavioral and physiological rhythms by masking them, and it is known that the sleep/wake circadian rhythm can be directly influenced by exercise (10, 29); 2) scheduled motor activity has an entraining effect on the circadian system of some animal species, such effects including accelerated reentrainment after LD shifting (25), a phase-response dependence of the shift induced by exercise (13), and the stable synchronization of animals without other environmental cues (9, 18); and 3) unscheduled motor activity influences the circadian system, affecting, for example, the free-running period of some rhythms (11, 26).

Our results confirm that spontaneous wheel-running activity affects circadian rhythmicity in rats. Most authors consider such an effect to be a feedback action of exercise on the circadian time-keeping system. However, as we reflected in the introduction, we shall restrict the term feedback to the regulatory loops in which pacemaker activity is modified by its own outputs. The masking influence of motor activity on other rhythmic variables controlled by the same pacemaker...
should not be considered as feedback. However, variables influenced by the masking can, in turn, act on the pacemaker(s) by feedback.

The present study is the first, to our knowledge, to report that exercise facilitates the establishment and expression of circadian rhythmicity under constant lighting conditions. It is known that exposure to prolonged periods of bright light reduces rhythm coherence and may even totally suppress circadian rhythmicity in rats (6, 7, 22). Three main hypotheses have been proposed to explain this effect.

1. Light puts an end to a single circadian pacemaker (7); in its place an ultradian rhythmicity appears, produced by a putative underlying ultradian oscillator (3, 12).

2. Prolonged LL exposure uncouples the components of a multioscillatory circadian system, thus enabling the different oscillators to run freely, producing an arrhythmic output pattern. The ultradian patterns observed in certain conditions would be the output of different oscillators (8).

3. Bright light uncouples the output of the system, while central oscillators function normally.

On the other hand, it has been described that bright light affects circadian rhythmicity according to the maturation state of the time-keeping clock. Thus perinatal exposure to LL determines the expression of a circadian free-running rhythmicity, whereas postweaning exposure determines an arrhythmic circadian pattern (4). These observations suggest that the circadian system may be organized in accordance to perinatal lightning conditions.

Our results showed that rats exhibiting a circadian rhythmicity (for instance by prior exposure to LD, exposure to constant DR, or early exposure to LL) can be induced to maintain their circadian feeding patterns under LL by the presence of running wheels. However, the presence of running wheels did not elicit any circadian pattern in rats with an arrhythmic feeding behavior. Motor activity prevented the loss of circadian rhythmicity but did not facilitate the emergence of this pattern in arrhythmic rats. According to these results, motor exercise, which is confined to a few hours during the day, might exert a positive feedback loop, periodically increasing the action of the pacemaker(s) on the feeding activity. Locomotor activity would maintain and strengthen the preexistent rhythmic patterns.
but would have no effect on an arrhythmic animal (Fig. 5).

An additional explanation of the effect of locomotor activity on the circadian feeding pattern may be the difference in the time taken to completely lose circadian rhythmicity in feeding and wheel-running activities after LL imposition. According to this hypothesis, the persistence of rhythmicity in locomotor activity could prevent the loss of circadian feeding rhythmicity by feedback and/or a masking effect, while in the absence of prior circadian rhythmicity, exercise cannot promote a circadian pattern. Such a partial dissociation between different rhythms in rats has been described previously (7).

Fig. 4. Diagrams of amplitude of circadian rhythmicity. Values are means ± SD for amplitude according to Fourier analysis of the records. In expts 1, 2, and 3, period analyzed corresponds to last days of the record, whereas in expt 4, A and B, the first days after weaning were analyzed. The period analyzed in each case is expressed in circadian time (CT). Lighting conditions (DR and LL) and wheel-running availability (WR+ and WR-) are indicated for every curve in the graphs. Values for a given experimental period that do not share a common letter are significantly different (P < 0.05).

Fig. 5. Feeding circadian rhythmicity appeared in every one of the phases of the 4 experiments. Circadian rhythmicity is represented with a line that oscillates according to the amplitude of the daily feeding rhythmicity observed in the results. LD, 12:12-h light-dark cycle.
Under DR, rats expressed a free-running feeding rhythm similar to that described in previous studies (5–7). However, the rhythms in WR+ rats were more pronounced than in WR−, which may be explained by a positive feedback effect of exercise on the circadian system. In a rhythmic animal, the concentration of wheel running in the active phase should increase the cyclic output of the SCN, strengthening the circadian feeding rhythm. However, it cannot be discounted that strong wheel-running rhythms may influence the expression of feeding rhythms by means of a masking effect (wheel running and eating cannot be carried out simultaneously), as occurs with sleep-wake rhythms in mice (10, 29). Although it is possible to speculate that the enhancement of feeding rhythms in animals allowed to wheel run could arise from an increased appetite due to additional energy loss, our data and results from others (14) showed that voluntary wheel running did not significantly increase food intake. Thus increased food intake cannot be responsible for the higher amplitude of the feeding patterns showed by WR+ animals.

When animals that had lost their circadian rhythmicity by previous exposure to LL were exposed to DR, they all progressively developed a circadian feeding pattern, according to previous experiments (7). However, this circadian pattern emerged more rapidly in WR+ than in WR− rats, which suggests that the effect of exercise not only buffers the changes in circadian organization (preventing or delaying the loss of circadian rhythmicity in LL) but also takes part in the emergence of a circadian pattern from an arrhythmic one. This last fact cannot be explained by a positive feedback effect because if exercise initially follows an arrhythmic pattern it should favor the maintenance of this arrhythmicity. However, the acceleration in the emergence of circadian rhythmicity suggests a more general effect of exercise on the circadian system. According to the multioscillatory hypothesis of circadian organization, exercise could enhance the strength of the coupling between different oscillators, facilitating a more rapid emergence of the circadian pattern (8). Once this circadian pattern is established, the positive feedback and the masking effect of exercise will contribute to a more solid circadian pattern in WR+ animals. It is not difficult to imagine that physical exercise could modify the coupling between neural oscillators by altering the levels of some neurotransmitters or hormones or by modulating tonic photic input to the oscillators. It is known that the serotonin content of the SCN is positively correlated with motor activity (26).

Finally, part of the effect of exercise on circadian rhythmicity in rat seems to be age dependent, as has been demonstrated in the case of bright light (4). The effect of wheel running on the circadian time-keeping system decreased with age, although this fact is difficult to dissociate from the effect induced by early bright light exposure.

The present results are endowed with a degree of practical interest. For example, if locomotor activity, in rats, induces a higher rate of coupling through a feedback effect, and a more pronounced circadian rhythmicity by means of a masking effect, physical exercise could improve the maintenance of circadian rhythmicity and, in turn, the health of people suffering from disruption of their circadian clock, such as the elderly or blind. Old people, particularly, tend to have a disrupted circadian organization of the sleep-wake pattern, with frequent naps during the day and a low quality of nocturnal sleep. A sustained increase in the diurnal activity, especially if adequately programmed, could contribute to the proper functioning of their circadian clock.

In summary, the present results demonstrate that physical exercise, even when unscheduled, prevents loss of circadian rhythmicity in LL-exposed rats and accelerates the emergence of circadian patterns in arrhythmic animals moved to DR. These results can be explained by taking into consideration a multioscillatory circadian system, in which exercise promotes stronger coupling, which is induced by a feedback effect that countermands the decoupling effect of LL.

**Perspectives**

The influence of feedback loops of rhythmic variables on their pacemakers is increasingly considered to be an important element in the physiology of the circadian system. Wheel-running availability exerts strong effects on feeding patterns in rats, including the prevention of the loss of circadian rhythmicity and acceleration of the emergence of circadian patterns in arrhythmic animals.

In addition, these results suggest new and interesting fields of study, such as 1) the plasticity of the circadian system in response to the perinatal exposure to LL or the possible plastic effect of physical exercise resulting from the availability of wheels early in the animals’ lifetime and 2) the physiological alterations associated with sedentariness in laboratory animals. In nature, wild animals develop an intense foraging activity that is normally obscured in laboratory studies of feeding rhythms, food intake regulation, and growth. This abnormal situation, which may alter many physiological processes, can be prevented by providing running wheels. 3) Finally, the feedback effect of physical exercise may be relevant for understanding circadian rhythm disorders in human beings such as the blind, elderly, or shift workers.

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