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Effect of Skeleton Photoperiod and Food Availability on the Circadian Pattern of Feeding and Drinking in Rats

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STRUBBE, J. H., N. J. SPITERI AND A. J. ALINGH PRINS. *Effect of skeleton photoperiod and food availability on the circadian pattern of feeding and drinking in rats.* PHYSIOL BEHAV **36**(4) 647–651, 1986.—Feeding and drinking behavior were measured in rats maintained under a 12:12 light-dark (LD) cycle or skeleton photoperiod (SPP). Feeding and drinking were closely associated during the normal LD cycle but under SPP conditions an increased feeding activity during the subjective day was not accompanied by an equivalent increment of water intake. This indicates a stronger coupling of drinking to the subjective night. A restriction of food availability to the subjective light phase did not cause an accompanying complete shift in drinking behavior. These results suggest that drinking is largely dependent on the influence of a circadian oscillator and this association is not disrupted by changes in feeding schedule. A change in food access to the subjective light phase caused partial but not permanent desynchronization between feeding and drinking behavior. Synchrony was reestablished within one day once food was available ad lib. Complete return to the original feeding and drinking patterning took 3 days. It is suggested that separate slave oscillators controlling feeding and drinking are governed by a hypothesized "master" circadian oscillator which remains definitely entrained to the original rhythm by the light pulses of the SPP condition.

Feeding behavior Drinking behavior Circadian pacemaker Skeleton photoperiod Food deprivation

FEEDING and drinking activity of rats fed on a normal laboratory diet shows a bi- or trimodal distribution over the dark phase with peaks at dawn and dusk [11]. Under normal conditions a very close temporal association exists between feeding and drinking so that 70–90% of daily fluid intake occurs around a meal [6,7]. Although these behaviors occur in very close temporal association the circadian rhythm of each behavior did not change significantly in the absence of the other behavior [8,9].

With a change in food availability or palatability to the daylight hours, several studies report that nocturnal wheel running and drinking activity remain mostly undisturbed [3,4] while others report a shift in behavioral and physiological variables associated with food intake [1,14]. The conflicting data may be due to feeding or palatability schedules used in these studies which may partially mask or override the entraining properties of the light-dark (LD) cycle. In a previous study we reported that when feeding behavior was restricted to the daytime only 17.5% of daily water intake was food associated compared with 71% during ad lib [11]. The circadian characteristics of temporal distribution of drinking activity and nest occupation were retained as well. These experiments further showed that although feeding and drinking may be causally related, they need not occur in close temporal association. However, it may be possible that light intensity had some aversive properties "forcing" drinking and most of the activities to occur in the dark phase despite feeding during the light phase. The aim of the present experiment is to investigate to what extent light is involved in the above mentioned dissociation between the behavioral expression of drinking and feeding behavior. The experiments were performed therefore under a LD regime employing skeleton photoperiod (SPP) and with food deprivation over the subjective night. This method with SPP leaves the endogenous component of light entrained behavior intact [10]. However, it is possible that shifting access to food to the subjective day phase does permanently reverse circadian control of food intake and eventually also of drinking behavior. In order to investigate this, we offered food ad lib after a long time of food access over the subjective day in a SPP situation.

METHOD

Animals and Housing

Six male Wistar rats, experimentally naive, about 4 months old and weighing 387–453 g were kept in two "climate" rooms, which were adequately screened from laboratory noise, 3 rats to a room. The rats were housed individually in Plexiglas cages. Each cage consisted of an outcage (40×40×40) with a dark perspex nest box (20×20×20 cm) attached to the outside of the cage. Entrance to the nest box was possible through a 4×4 cm opening. The nest box contained wood shavings for bedding, whereas the outcage had a rigid wire mesh floor.

Except during the food restriction periods, food pellets

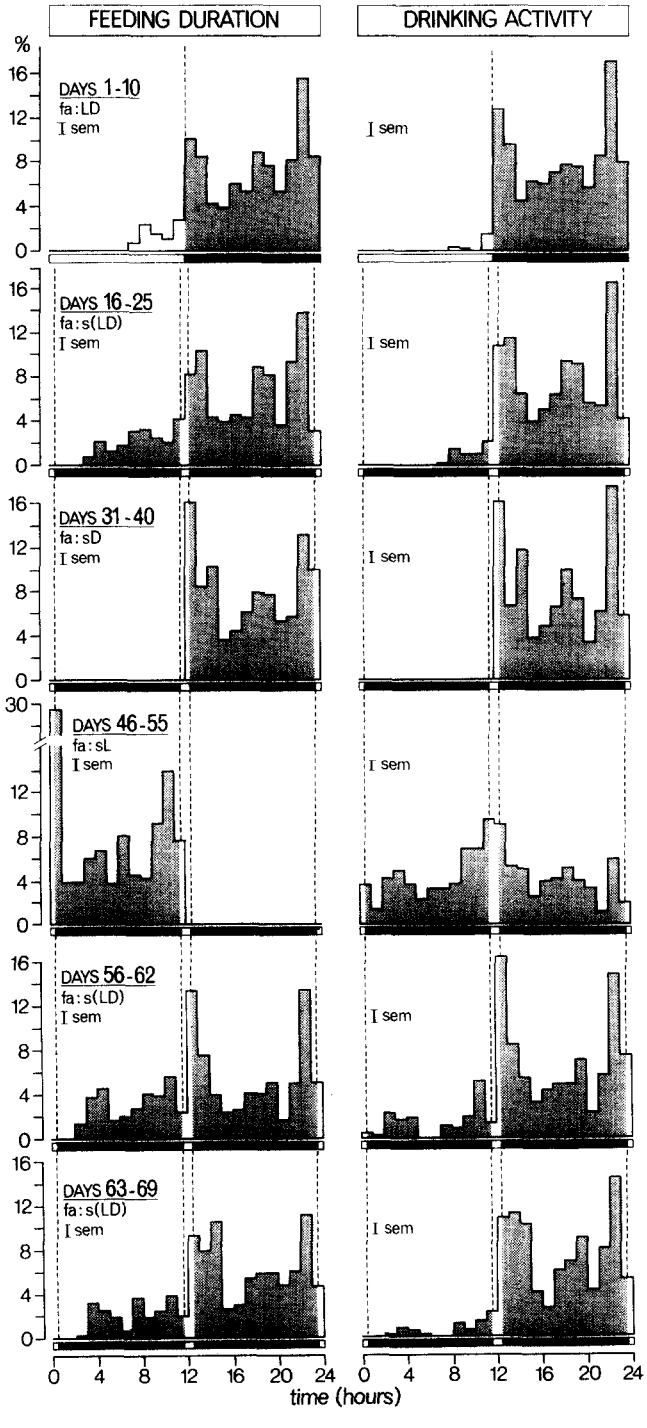

FIG. 1. Percentage distribution of feeding and drinking behavior over the LD cycle and during skeleton photoperiod of male rats with normal access to food and water (days 1–25 and days 56–69) and with food access either during the "subjective" night (days 31–40) or the "subjective" day (days 46–55). fa: food availability, s: subjective. SEM is the averaged standard error of the mean.

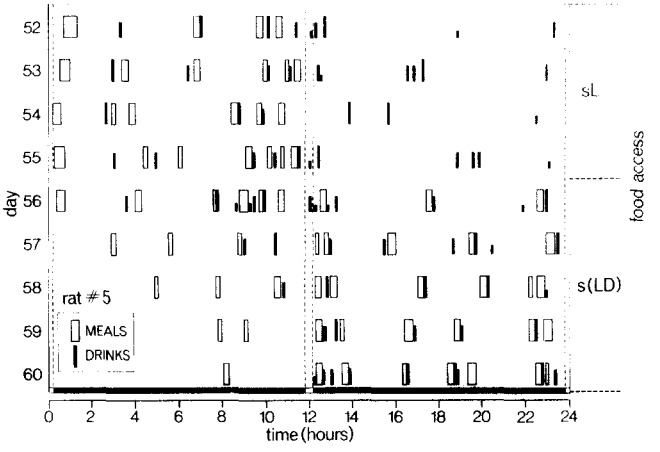

FIG. 2. Diagrammatic representative meal pattern for one rat during 9 days from fa:sL to fa:sLD on day 56. A larger black bar means a larger drinking bout.

(Muracon, Trouw, The Netherlands) in hoppers were freely available. Water was always freely available. The food hopper and water bottle were situated opposite each other, 40 cm apart, at the distant ends from the nest box. Food hoppers and water bottles were filled regularly, about two times per week and at different times in the light phase of the LD cycle. Routine maintenance of the cages was carried out during these times. Lighting in each room was provided by three overhead 40 W daylight type fluorescent tubes. Light intensity inside the cages was the same for all rats and varied between 4 $\mu W \cdot cm^{-2}$ at cage floor level to about 20 $\mu W \cdot cm^{-2}$ at the level of the food hopper. Light intensity in the nest box was very low (1 $\mu W \cdot cm^{-2}$). Light intensity measurements were made with an UDT model 40 optometer using a radiometric filter and a foot candle diffuser. Room temperature was thermostatically controlled at 21°C. Relative humidity was constant at 60%.

Recording

Feeding and drinking behavior were recorded continuously throughout the experiment on a 20-channel Esterline Angus Event Recorder.

Food Intake

The recording method of feeding activity has been described elsewhere in detail [11]. Briefly, the gnawing and biting of food pellets from food hoppers through stainless steel bars caused the food hopper to swing slightly. This movement produced an electrical signal causing a pen deflection on the event recorder. Most of the spillage was collected in an undertray attached to the hopper. The daily amount of spillage collected ranged from 0.05–0.2 g. Access to food was restricted by means of an automatic, horizontally-sliding door situated in front of the food hopper.

Water Intake

Drinking activity was recorded by means of an L-shaped stainless steel pedal situated below the water bottle with a vertical lip just in front of the drinking spout. A brass hood was mounted above the drinking spout. Depression of the pedal exposed the drinking nipple and activated a micro-

TABLE 1

MEAN DAILY VALUES OF FOOD (g) AND WATER INTAKE (ml), WATER/FOOD RATIO AND BODY WEIGHT (g) DURING THE SUCCESSIVE CONDITIONS OF THE EXPERIMENT

	fa:LD	fa:s(LD)	fa:sD	fa:sL	fa:s(LD)	fa:s(LD)
Food intake	24.2 ± 1.5	24.5 ± 1.5	23.2 ± 1.6	20.1 ± 1.2*	24.2 ± 1.8	24.3 ± 1.5
Water intake	25.7 ± 2.1	25.6 ± 2.1	25.2 ± 2.6	26.2 ± 4.1	24.2 ± 2.4	25.1 ± 2.2
Water/food ratio	1.05 ± 0.03	1.04 ± 0.03	1.08 ± 0.06	1.28 ± 0.14	0.99 ± 0.03	1.03 ± 0.03
Body weight	441	451	460	469	480	489

Mean ± SEM.
*$p<0.01$.
fa: food availability; s: subjective.

switch. Visual observations of rats drinking correlated well with recordings [11]. Rats kept the drinking pedal depressed only while drinking.

Meal and Drink Definitions

An intermeal interval of 15 minutes was adopted in defining periods into individual meals. The rationale for adopting this criterion has been presented elsewhere [13].

Drinking bouts were divided into the following two categories: (1) meal-related drinking, all drinking bouts occurring up to 15 min *prior* to, *during*, and up to 15 min following a meal; (2) non-meal-related drinking; *separated* from meals by at least 15 min.

Experimental Procedure

The experiment lasted 13 weeks and consisted of the following periods: The rats were given a 3 week habituation period in the cages with food and water available ad lib on a normal light dark cycle (LD 12:12). The last 10 days of the habituation period were considered as control for the experimental period (days 1–10). Following this period, a skeleton photoperiod was introduced in which two 30 min light pulses were presented each day during periods when light transitions would occur during a normal light-dark cycle. Food and water were still available ad lib. This period lasted for two weeks, the last 10 days being taken for experimental purposes (days 16–25). This skeleton photoperiod was maintained for the following weeks. In the first two weeks food was restricted to the original dark phase, the last 10 days being taken for experimental purposes (days 31–40). In the following two weeks, food was made available (fa) to the rats only during the "subjective" light phase (fa:sL; s for subjective). The subjective light phase is the phase where it was light in the previous condition without skeleton photoperiods (days 1–10). Again the last 10 days of these periods were considered experimental days (days 46–55). Thereafter food and water were freely available again for two weeks (days 56–69). Body weight was measured once per week.

Data Analysis and Presentation

Food intake. For each rat the mean time spent on feeding during 60 min in all hours of the 24 hr LD cycle or the skeleton photoperiod was calculated per week and expressed as a percentage of total feeding duration. The means and averaged standard errors (SEM in Fig. 1) for 6 rats were presented as histograms. Averaged standard errors of the means were presented since these were small and very similar, both for feeding duration and drinking activity.

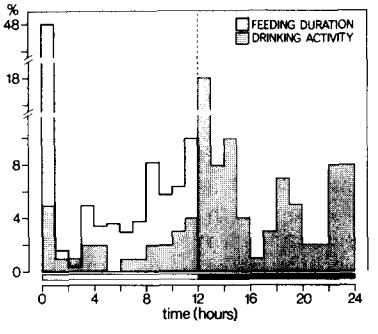

FIG. 3. Percentage distribution of feeding behavior over the LD cycle with food access during the light phase.

Water intake. The following method was adopted in obtaining a measure (index) of drinking activity: Drinks shorter than 1 min were scored as 1; drinks shorter than 3 min and longer than 1 min were scored as 2; and drinks longer than 3 min were scored as 3. Individual tests on rat drinking behavior showed that the drinking rate for each rat remained remarkably constant throughout the experiment. These tests also showed a strong agreement between the amount of water consumed and the drinking index.

RESULTS

Changes in Feeding and Drinking Patterns

Figure 1 shows the distribution of feeding and drinking over the LD cycle and during a skeleton photoperiod of male rats with normal access to food and water (days 1–25 and days 56–69), and with food restricted either to the "subjective" night (days 31–40) or the "subjective" light phase (days 46–55). Feeding and drinking were predominantly bimodally distributed during the dark phase with a prominent peak in the beginning (dusk peak) and another towards the end (dawn feeding peak) of the night (Fig. 1, days 1–10). A third, smaller peak was also evident towards the middle of the dark phase. Drinking was more nocturnal than feeding, respectively 99% versus 92%. The water to food ratio was 0.16 during daytime and 1.12 during nighttime. With the introduction of the skeleton photoperiod (fa:s(LD); days 16–25) rats showed earlier drinking and feeding activity during the "subjective" light phase. Drinking, however, was

again more nocturnal than feeding, respectively, 95% versus 81%. The water to food ratio was 0.28 during daytime and 1.13 during nighttime. In general, the feeding and drinking patterns were very similar to the ad lib situation. A similar pattern, but without any drinking behavior in the subjective light phase was observed when food deprivation occurred during that time (fa:sD, days 31–40). The "dusk" peaks of feeding and drinking activity increased from about 10% to 16% during this period. Particularly, the "dawn" peak in drinking activity remained large and pronounced.

When food was restricted to the subjective light phase (fa:sL; days 46–55) feeding activity was bimodally distributed over the sL phase, similar to the normal feeding pattern. However, the "dawn" peak showed a large increase, and feeding in this period amounted to almost 30% of the total feeding activity. This occurred during the first hour after the "dawn" light pulse. A small increase in feeding was observed during the middle of this phase, followed by a feeding peak at the end. This end peak in fact was very similar to the peak seen in the ad lib situation at "dawn."

In the fa:sL condition the drinking pattern differed from that of feeding. The changes in drinking activity are clearly shown in Fig. 1. About 50% of the total drinking activity occurred during the sD phase, with a lot of drinking directly preceding and following the dusk light pulse. This "dusk" drinking still represents a substantial amount of drinking in the night phase. It is interesting to note that drinking in the first hour after the dawn light pulse was low, and sometimes no postprandial drinking was seen associated with the large meal following the dawn light pulse (see also Fig. 2).

On return to fa:s(LD) (Fig. 1, days 56–62 and 63–69) both feeding and drinking activity quickly returned to the distribution observed in days 16–25.

The selection of meal and drink patterns of one rat presented diagrammatically in Fig. 2 was typical of all rats. The patterns show several interesting features which merit consideration. First, most of the drinking in the fa:sL situation during the subjective light phase was preprandial in the beginning and prandial or postprandial at the end. Second, Fig. 2 also shows some persistence of the nocturnal drinking pattern when food was restricted to the sL phase. Finally, when food was available ad lib again, the first "reassociation" or resynchronization between feeding and drinking patterns deserves consideration (Fig. 2, days 57–60). Also the almost total disappearance of drinks from the sL phase, and the shift in meal patterning from the sL phase towards the sD phase, deserves attention. It should be noticed that these changes occurred very quickly and that the normal pattern is already attained within 3 days. Note for example the three meals during the first day when food was available in the sD period again (day 56). The timing of some of these meals and drinks shows remarkable constancy from day to day.

Food, Water Intake and Body Weight

Daily means for food and water intake are presented in Table 1. There was no change in water intake during any of the conditions, whereas food intake showed a significant decrease during the fa:sL condition (paired t-test: $p<0.01$). This did not result in a drop in body weight. During this condition water intake showed a small but non-significant increase. This is best reflected in the water/food intake ratio.

DISCUSSION

The present results show that under skeleton photoperiod (SPP) an increase in feeding activity of 11% occurs during the subjective daytime compared with the LD situation. Feeding and drinking are closely associated in time during the LD situation. However, water to food ratio is much lower during daytime than during nighttime in the LD as well as in the SPP condition. The increased feeding activity in the subjective day during the SPP condition is not accompanied by an equivalent increment of water intake in that period (11% for feeding and 4% for drinking), indicating a stronger coupling of drinking behavior to the subjective night. When rats are deprived of food over the subjective night in the SPP condition, feeding activity during the subjective day period shows a pattern more similar to that in the ad lib condition than the pattern seen in a previous study using a normal day-night cycle, with food deprivation during night time [11] (Fig. 3). In the latter condition rats lose body weight and eat less than in the SPP condition where no decrease in body weight was seen, indicating a strong suppressive influence of light in the behavioral expression of feeding. The large increment in food intake in the first hour (Fig. 3) which is higher in the normal LD condition (48%) than in the SPP condition (30%), may be explained by the larger energy deficit. There is a small but significant decrease in food intake in the fa:sL condition compared to the fa:sD condition although body weight was not affected. Since the external condition (light intensity) is the same it is suggested that besides light the circadian oscillator also induces a depression in food intake in the fa:sL condition. Because some energy-cost activities (i.e., drinking behavior) remain in the subjective night, the energy deficit in the fa:sL condition may be larger than in the fa:sD condition, where no drinking was performed during the subjective day. This may be one reason why the first hour intake (30%) is higher in the fa:sL than in the fa:sD condition (16%). Another explanation may be the stronger stimulating influence of a circadian oscillator on feeding during dawn than during dusk [5,12].

In the fa:sL condition drinking behavior increased more during the subjective day than during the light period in a previous study with a normal light-dark cycle [11]. This confirms the strong suppressive properties of light on drinking [10]. Drinking in the fa:sL condition was concentrated around the light pulse of the original dusk period. It is remarkable that rats drink a relatively small amount after ingesting a large meal just after the "dawn" light pulse. These observations may indicate that drinking behavior is under command of a circadian oscillator acting during the dusk period [10], while eating may be a stimulated by an oscillator which is more active during the dawn period [5,12].

After returning to the ad lib condition peak activities of feeding activity shift very rapidly back to the subjective dark phase the normal pattern being reached already after 3 days. Food associated drinking also shows a rapid shift and is again largely restricted to the dark phase. A greater nocturnality in drinking than in feeding behavior is also found by others [2,10]. As soon as the ad lib condition is returned, the rats take meals at the beginning, middle and end of the subjective night while one might expect that they would have eaten until satiation was achieved in the previous subjective daytime so that they were able to bridge the subjective night energetically. Nevertheless after returning to the ad lib situation a relatively high level of feeding behavior remains in the subjective light phase. Similar observations were made in rat dams after weaning, which also eat large amounts of food during the light phase during lactation [13].

In summary, drinking is more dependent on the circadian

pacemaker than feeding which becomes apparent when (1) drinking is more nocturnal than feeding both during LD and also in the SPP condition, and (2) a change of food access to the subjective light phase causes partial but not permanent desynchronization between feeding and drinking behavior. Synchrony is established very quickly (within one day) again once food is available ad lib. Complete return to the original feeding and drinking patterning took 3 days. Therefore, if one can distinguish two separate slave oscillators governing feeding and drinking behavior [2,10] this study shows that a hypothesized "master" oscillator remains definitely entrained by the light pulses, otherwise one would have seen a complete reversal in circadian behavioral expression.

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