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Food anticipatory yawning rhythm in the rat

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Abstract. The effect of feeding schedules on the daily rhythm in spontaneous yawning activity was studied in high yawning (HY) Sprague-Dawley rats. If the animals are fed ad libitum and changed from a standard 12-12 light-dark (LD) illumination regime to constant light (LL), the normal predark circadian peak in yawning disappears, to be replaced, after 3 weeks, by two or more ultradian smaller peaks in yawning frequency. Restriction of food availability to 2-2:30 regular hours of the day, in rats under LL conditions, leads to the appearance of a significant preprandial (food anticipatory) peak in yawning. A similar eating-fasting daily cycle of 2-22 h in rats under LD conditions determines the disappearance of the pre-dark peak in yawning activity, and a significant shift in higher yawning frequency towards the couple of hours preceding food availability. This result suggests that restricted feeding is more potent than the LD transition in the entrainment of the daily rhythm in yawning activity.

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INTRODUCTION

A brief communication in this same journal (Anias et al. 1984) described the existence of circadian variations in the frequency of spontaneous yawning in the rat. The observations were made on a line of Sprague--Dawley rats, genetically selected to obtain a higher incidence of yawning (HY rats) (Urba-Holmgren et al. 1990). In that report an effort was made to correlate diurnal changes in yawning frequency with circadian variations in activity (as reviewed in the literature) of the neurotransmitter systems that had been postulated as participating in the central control and regulation of yawning (Anias et al. 1984). Since yawning frequency was highest during the last light hour, both under natural and artificial light-dark (LD) illumination schedules, it was suggested that the LD transition might be the "primary synchronizer" of the circadian rhythm of yawning. But that suggestion was not supported by a formal differentiation between circadian rhythms proper, which are capable of free--running in absence of the entraining stimuli, and daily or diurnal rhythms, which "are induced and dependent upon rhythmic occurrence of environmental events" (Moore 1980). Nor had the possibility been explored that yawning behaviour might be entrainable to "secondary synchronizing" stimuli, as restricted food or water availability which had been proved to be important synchronizers of several behavioural rhythms (Krieger 1974, Edmonds and Adler 1977, Krieger et al. 1977, Morimoto et al. 1977, Takahashi et al. 1977, Krieger and Hauser 1978, Sulzman et al. 1978, Morimoto et al. 1979, Phillips and Mikulka 1979, Boulos 1980, Moore 1980, Coleman et al. 1982, Dhume and Cogate 1982, Inouye 1982, Honma et al. 1983, Mistlberger and Rechtschaffen 1984) (for recent reviews see Rusak 1981, Takahashi and Katz 1982, Hiroshige 1984, Turek 1985, Rosenwasser and Adler 1986). The purpose of the present work is to study spontaneous yawning behaviour in rats maintained under continuous illumination, and examine if food availability, regularly restricted to 2 or 3 daytime hours can entrain a rhythmic variation in yawning, both in rats under constant light, and in animals under normal 12-12 LD cycles.

METHODS

This study is based on the observation of several groups of male Sprague-Dawley rats (46 animals in total), from the 8th, 9th, 13th and 16th generations of the HY subline, genetically selected at our Animal House. Their general housing and feeding conditions and the method we use for the observation of yawning have already been described (Anias et al. 1984, Holmgren et al. 1985).

At the preliminary stage we observed yawning for 24 h a day in sessions of different durations irregularly distributed throughout several days. But as this procedure seemed to introduce unwanted variability, in each experimental condition yawning was at the end monitored during an uninterrupted session of 24-26 h. Finally, because of the inadequateness of averaging data from different animals in a free running condition, we introduced a balanced experimental design in which each animal was its own control and we monitored yawning continuously during 56-60 h on two occasions, separated by 22 days.

Different group of animals were studied exclusively under one of the following conditions (A-D):

A. Constant light and ad libitum feeding

The observation of yawning behaviour, under constant illumination (LL), was initially done in twelve animals (two rats per cage) from the 8th and 9th generations of the HY subline. At the age of 2 months they were transferred from the Animal House to a laboratory observation room of 18 m^2 permanently illuminated with two 80 watts 2.20 m long fluorescent tubes from a 4 m high ceiling. The room was not exclusive for the experimental animals: during the day it was randomly used for observation of other animals and study purposes. Although silence was recommended, slight noise and personnel traffic were inevitable between 08:00 and 19:00. After 3 weeks of adaptation to the LL regime, with food and water available ad libitum, yawning occurrence was monitored continuously during 24 h by trained couples of observers replacing each other every 2 h. Finally, two additional groups of four rats each, from HY F13, after the same period of adaptation to LL, were subject to yawning monitoring in uninterrupted observation sessions of 24 h.

B. Restricted daytime feeding in animals under constant illumination

For this purpose the rats were changed to clean boxes every day, at irregular intervals before giving

them their daily ration of standard laboratory rodent pellets (Purina), placed directly on the layer of wood shavings covering the floor of the boxes. During the adaptation period to restricted feeding, which lasted 18 days, the animals were sometimes weighed before and after eating, in order to estimate their daily food intake. Excess of food was removed from the cages at the end of the feeding time. The animals had free access to water over 24 h. Two groups of 4 rats each and one of 6 animals were subject to three slightly different feeding and observation schedules: one group had food available during 3 h (14.00 to 17.00) and the observation sessions took place over 30 days after the initial period of adaptation to this regime; the second group had a restricted feeding time (RFT) of only two hours (12.00 to 14.00),the observations being made during 45 days after the adaptation period; the third group had food available during two and a half hours (08:00 to 10:30) and was observed continuously in a single session of 26 h, which began with the animals eating their daily ration of food pellets, and ended with the rats fasting until 10:00 next day.

C. Restricted feeding time in rats under a LD schedule

A group of six two month-old HY rats, from the 9th generation, housed in two cages with three animals per cage, were adapted to a LD cycle (lights on from 7:00 to 19:00) with food available between 9:00 and 11:00. Once adapted to this regime for 3 weeks, the rats were continuously observed and yawning monitored during 26 h, starting from 9:00 one day to 11:00 next day.

D. Balanced experimental design with animals being their own controls

In these experiments, we used six two month-old HY-males from generation F16, randomly distributed in two groups of three animals each and treated under continuous illumination according to the following feeding schedule:

Animals	Phase 1	Phase 2
group 1	ALF (18 days)	RFT (22 days)
yawn monitoring	60 h	52 h
group 2	RFT (18 days)	ALF (22 days)
yawn monitoring	60 h	52 h

ALF, ad libitum feeding; RFT, restricted feeding time (12:00-14:00).

E. Fasting experiments

After completing the observation of yawning along 24 h of the day, some of the animal groups fasted during 48 to 96 h, with yawning monitored several times during that period in order to study the evolution of spontaneous and drug induced yawning under fasting conditions. These experiments will be described in full separately.

STATISTICS

For the first three experimental designs, the results were analysed using non parametric directional statistics (Batschelet 1981). For each animal we determined the hour-interval with the highest number of yawns, with the middle point of this interval expressed as an angle of a unitary circumference. With a single vector from each animal and using all the animals that had received the same treatment, we calculated the mean directional vector (r) and its angular deviation (s). To prove statistically significant directionality for each treatment, we applied Rayleigh's test of hypothesis (Batschelet 1981). For unimodal samples the length of the mean vector is a measure of concentration of the data. When vectors point in divergent directions the value of r will decrease from 1 to 0.

For the balanced experimental design, the number of yawns/hour from each animal under each treatment was analysed using time series technique (Kullback 1968). To describe the evolution of the behavioural pattern we obtained the autocorrelations and the partial autocorrelations. Autoregressive-Moving Average (ARIMA) models were estimated for each time series. As a complementary point of view,to look for periodicities we applied spectral analysis.

RESULTS

Free-running yawning behaviour

When yawning behaviour was studied in rats living under LL, the significant peak in yawning activity that precedes the LD transition, described previously (Anias et al. 1984), did not appear. Instead, as illustrated in Fig. 1, several peaks of yawning above average frequency appeared at different hours of the day for different animals, conveying the impression of a disorganized daily yawning rhythm. Since their highest
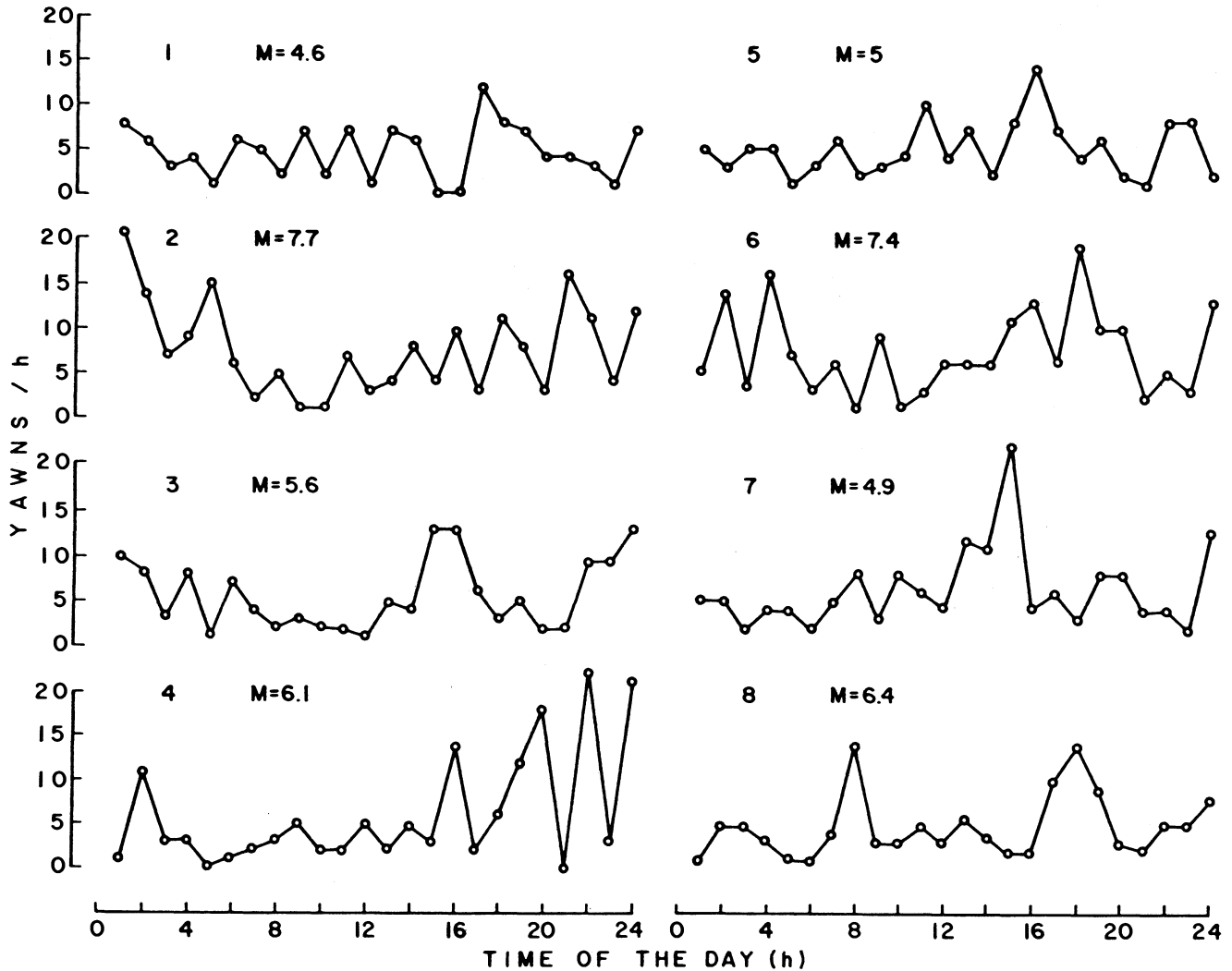

Fig. 1. Yawning frequency in rats after 3 weeks adaptation to constant light and ad lib. feeding. Individual curves from 8 animals in which yawning was monitored continously during 24 h. M, mesor values.

Fig. 2. Directional statistics graphs representing yawning frecquency in rats under different experimental conditions. For each animal the middle point of the hour interval with highest number of yawns was expressed as an angle (vector) of a unitary circumference. Mean directional vectors (r) and their angular deviations (s) were calculated for each experimental condition: A, Under LL and lib. feeding (ALF); B, LD illumination schedule and ALF; C, LD and restricted feeding time (RFT); D, LL and RFT (3 h); E, LL and RFT (2.5 h);F, LL and RFT (2 h). Outer black semicircles in B and C indicate the dark period of the day. White areas drawn on the unitary circumferences in C, D, E and F represent the respective periods of RFT. Numbers directly in front of some vector arrowheads indicate number of animals with most frequent yawning at that same hour interval.
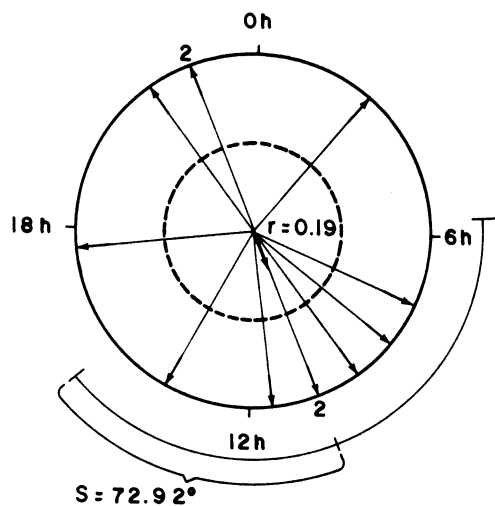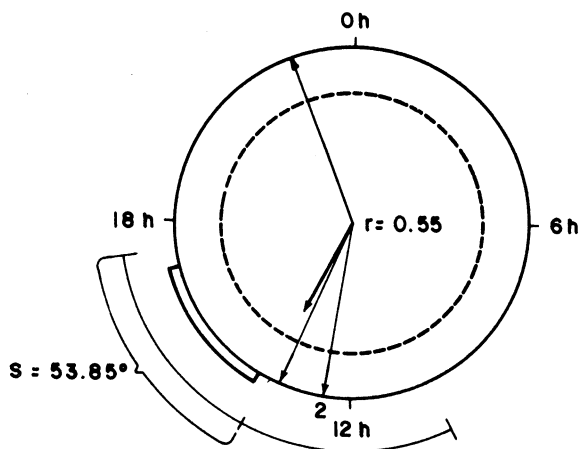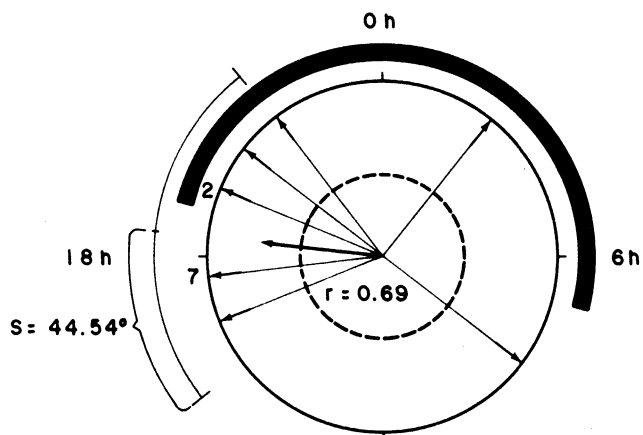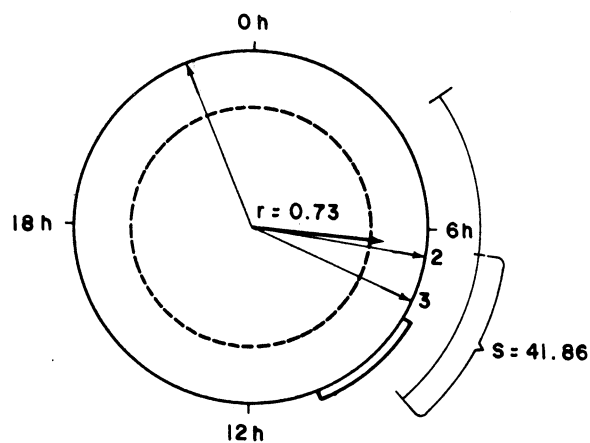
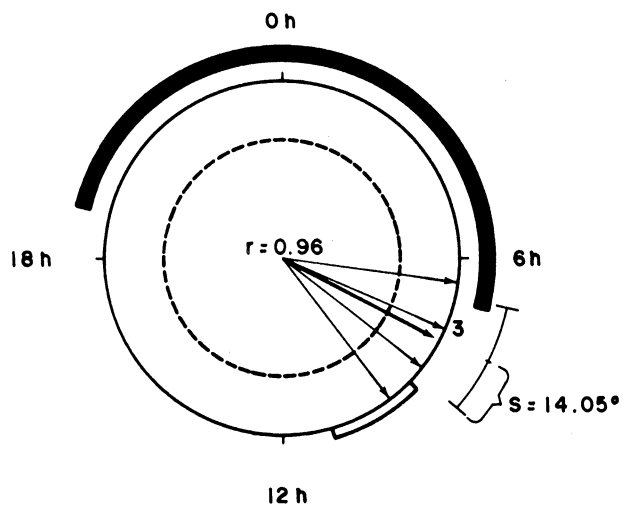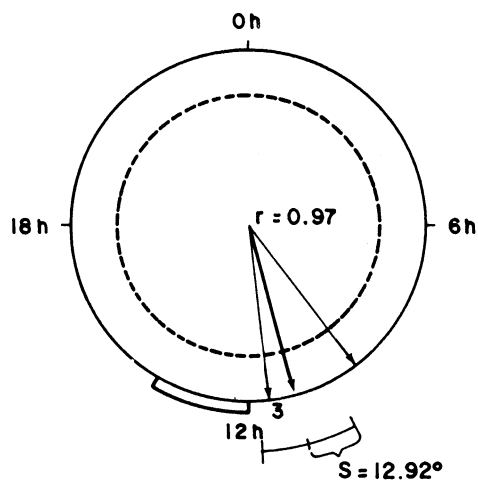yawning frequency hour intervals, represented by vectors, pointed to very different hours, the length of the mean vector ($r = 0.19$) was very short and its angular deviation ($s = 72.92°$) large (see Fig. $2A$). Rayleigh's test indicated that there was no statistical evidence of directionality.

Restricted feeding in animals under LL

The imposition of a regular restricted feeding time (RFT) of 2-3 h/day to rats under constant light, determined the appearance of an anticipatory peak in yawning activity during the hours preceding food availability. The peak was the higher the shorter was the period of access to food, perhaps attributable to better synchronization of the individual animals' feeding behaviour in relation to the time of feeding (compare the length of the mean vectors and the angular deviations in Fig. $2A,E$ and F). The appearance of this anticipatory peak in yawning in relation to the initiation of RFT is statistically significant only with 2-2.30 h RFT (not with 3 h), whatever the time of day in which food was made available.

Restricted feeding in rats under a LD cycle

In this experiment, done in two groups of 3 animals each, with lights off from 19:00 to 7:00 and the rats fed from 9:00 to 11:00, even if the two potential synchronizers, food availability and LD transition, were present, yawning activity was highest preceding the feeding period (Fig. $2C$). This preferred period for yawning is significant when analyzed with Rayleigh's test (Batschelet 1981).

These results point to the relatively higher power of the food synchronizer in relation to the light-dark transition, but do not implicate nonexistence of the latter synchronizer. In animals under ad libitum feeding and a LD schedule (data taken from Anias et al. (1984) the mean directional vector points to 18.24 h, anticipating darkness (see Fig. $2D$). Even if directionality is less pronounced it is statistically significant.

Balanced experimental design in rats under LL

FIRST PHASE

As expected, group 1 of free-fed animals exhibited an apparently disorganized rhythm of yawning beha-

viour, with several peaks in yawning distributed at different hours of the day (Fig. $3A$). No autocorrelations corresponding to a period near 24 h were significant in this group, but in two animals the autocorrelations with periods of 6 and 7 h respectively were significant. Animals from group 2 (under RFT) clearly showed yawning peaks in the hours preceding feeding time (Fig. $3C$), the correlograms showing significant autocorrelations of 24 h periods in two of the three animals, the third being on the borderline of 95% significance.

SECOND PHASE

After 22 days of adaptation to the inverted conditions: group 1 with RFT and group 2 with ALF, yawning changed its diurnal pattern (Figs. $3B$ and D) to that expected for the new conditions. Nevertheless, in one animal of group 2 a residual effect on yawning of the former period of RFT seemed to persist, because its partial autocorrelation with a period of 24 h was still significant.

In general, the time series corresponding to animals under RFT, independently of the order in which this condition was studied, could be represented by an ARIMA $(0,1,1)_{24}$. For the series from the animals subject to ALF no model could be adjusted. Some comments related to the behaviour of rats under restricted feeding are noteworthy. Yawning of rats during the feeding hours is sensibly reduced - the rats eat and do not yawn. Autocorrelations and the estimated spectral density functions could reflect a 24 h seasonality strongly influenced by these observations and not by the presence of a circadian rhythm. Following the suggestions of an anonimous referee (of an early version of this article), we substituted the yawning data during the periods of feeding by randomly generated data with the mean and variance equal to those data from the remaining time. Twenty four hour periodicity persisted. We also resorted to eliminating observations in feeding periods and, to make the results comparable, the same was done with animals under ALF. With and without these observations, the same conclusions were obtained, indicating that their influence in the analysis was negligible. That is, under RFT, the pattern of autocorrelation and the spectral density estimates clearly showed a one-day seasonality; under ALF, the animals exhibited an apparently disorganized rhythm of yawning behaviour.
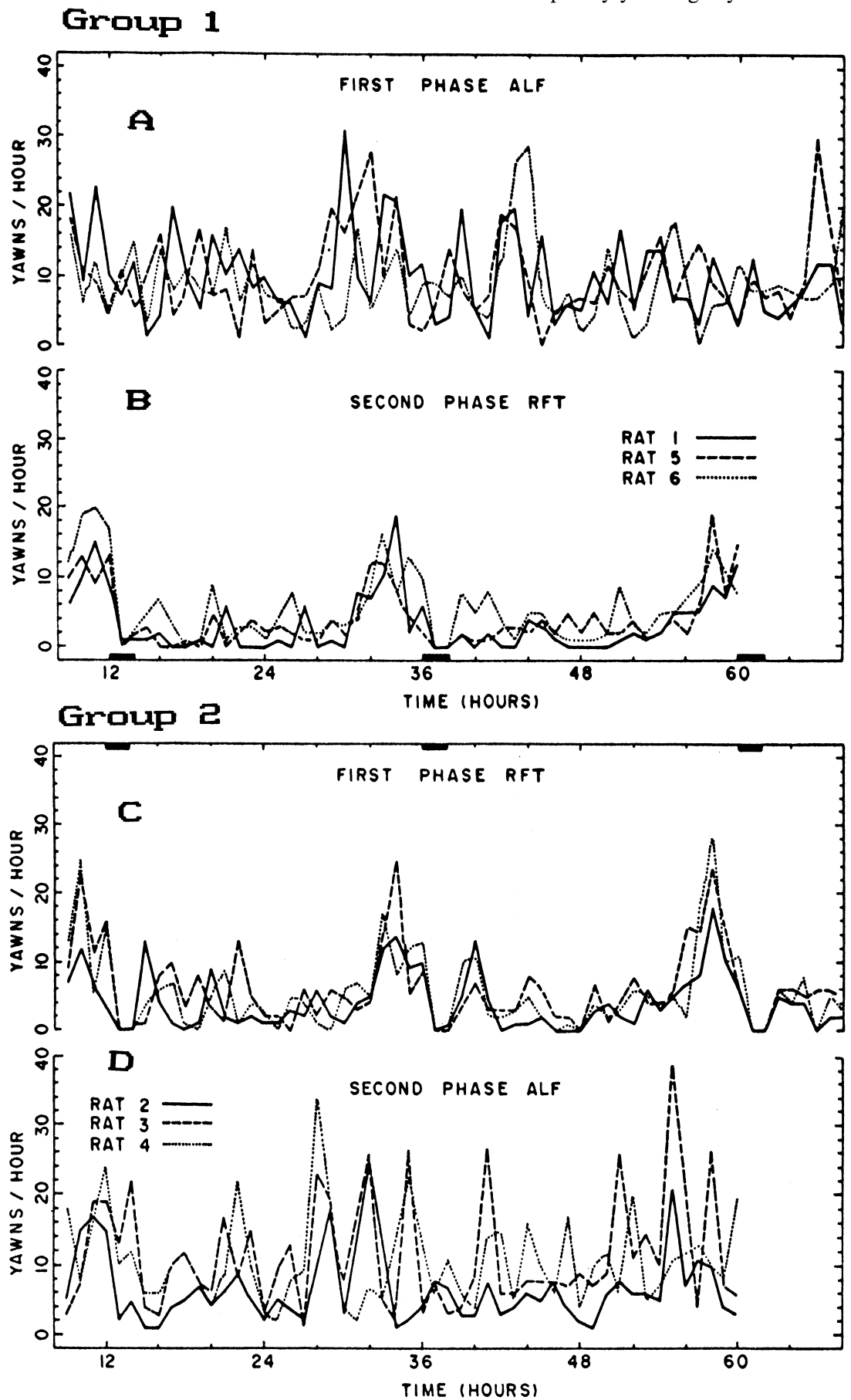
Group 1

Fig. 3. Comparison of yawning in rats (n = 6) under restricted feeding time (RFT) or ad. lib feeding (ALF). Balanced experimental design with yawning monitored continuously during 60 hours (1st phase), and 52 h (2nd phase). Both phases were separated by 22 days of adaptation of the two experimental subgroups to the changed feeding conditions. For more details see text.

Effect of fasting
on yawning activity

In rats adapted to a single daily feeding period of a couple of hours, yawning was practically nil during that period. The animals concentrated most of their activity on eating, with short intervals for drinking or, when being dominant rats, for snatching food pellets from their cage partners and trying to store them in their preferred cage area. In these feeding conditions the rats managed to conserve their 2 months-old bodyweight, age at which the adaptation to food restriction began. A 2 h daily feeding period obviously implies a regular daily fasting period of 22 h. If fasting was prolonged, by not giving the rats their expected daily ration, spontaneous yawning frequency decayed, and practically disappeared if the animals were allowed to fast for 3 or 4 days.

DISCUSSION

The initial purpose of this work was to establish whether the daily rhythm in yawning behaviour described in the rat, with its peak in frequency preceding the LD transition (Anias et al. 1984) could be properly considered as circadian, i.e. as an endogenous rhythm governed by a circadian pacemaker, entrainable to the LD transition and capable of free-running in its absence. In our experiments with rats adapted to live under constant light, and fed ad libitum, yawning activity exhibits several peaks during the 24 h (Fig. 1), as if under aperiodic environmental or internal underlying influences, rather than under the regular control of a circadian pacemaker. The vectors representing maximal yawning hour intervals of the different animals over 24 h seem to be randomly distributed (Fig. 2A).

The loss in the diurnal rhythm of yawning behaviour in rats exposed to constant illumination while fed ad libitum does not necessarily mean that this rhythms is not endogenous. Several well documented circadian rhythm, as those of plasma corticosteroid concentration, body temperature levels and food consumption, which in rats are entrainable to the LD cycle, also tend to disappear under constant dim light (Takahashi et al. 1977, Honma and Hiroshige 1978a, 1978b, Morimoto et al. 1979) prolonged for more than one month.

The importance of the time of feeding in modifying behavioural rhythms has been known for a long time. Honma et al. (1983) recalled that already in 1922 Richter described an increase in locomotor activity in rats before the time of feeding. Restricted periodical feeding has more recently been used by many authors and in different animals to entrain other biological rhythms: locomotion, running activity, body temperature, drinking and urinary excretion, adrenocortical activity, etc..(Krieger 1974, Edmonds and Adler 1977, Krieger et al. 1977, Morimoto et al. 1977, Takahashi et al. 1977, Phillips and Mikulka 1979, Boulos and Terman 1980, Moore 1980, Coleman et al. 1982, Inouye 1982, Honma et al. 1983, Hiroshige 1984, Mistlberger and Rechtschaffen 1984, Shiraishi et al. 1984). Our experiments with HY rats under constant light and only one regular daily meal, show that after 3 weeks' exposure to this regime, a significant peak in yawning activity anticipates the time of food availability.

In captive African lions and mandrils in a Zoo, subjected to regular feeding times, Baenninger (1987) has recently described a clear peak in yawning during the last hour before the animals received their food. But this does not necessarily mean that yawning might be a sign of hunger, as suggested by Barbizet (1958).

If fasting was prolonged for more than 24 h spontaneous yawning diminished, and practically disappeared if the animals fasted 3 or 4 days. Under these last conditions even apomorphine-induced yawning was completely blocked (Anias and Holmgren, unpublished results).

As to the relative potencies of the L-D transition and the restricted meal time as entrainers of the yawning daily rhythm, the experiment illustrated in Fig. 2C seems quite convincing: the peak in yawning just before dark is absent. These results confirm the opinions of other authors that the time of food presentation (Edmonds and Adler 1977, Krieger and Hauser 1978, Sulzman et al. 1978) or of water availability (Dhume and Cogate 1982) may be more potent synchronizers than the L-D cycle, in relation to several rhythmic physiological or behavioural variables. Since rats under a LD and free feeding schedule eat mostly during the early dark hours, this eating habit might be permanently reinforcing the tendency for a pre-dark peak in yawning to appear, making the L-D transition look like a stronger synchronizer than what it really is.

Yawning has commonly been considered to be related to drowsiness preceding or following sleep (Barbizet 1958). But increased yawning behaviour, anticipatory to the restricted feeding time in rats, seems

to coincide with a state of alertness of the animals, with increased locomotor activity (Phillips and Mikulka 1979) or wheel running (Edmonds and Adler 1977, Coleman et al. 1982, Honma et al. 1983) and higher plasma levels of corticosteroids (Krieger 1974, Morimoto et al. 1977). Food "expectancy" might, in a certain way, be considered as a sort of psychological stress or situational anxiety, condition which recently, in a very brief editorial comment (Shader and Greenblatt 1985), has been mentioned to be positively correlated with yawning in humans.

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