

Computational and Entrainment Models of Circadian Food-Anticipatory Activity: Evidence From Non-24-Hr Feeding Schedules

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Rats anticipate a daily meal, provided that meal onset intervals are within a circadian (22–31 hr) range. Food-anticipatory activity (FAA) has been interpreted as evidence for a food-entrained circadian pacemaker or for a computational process that uses stored representations of pacemaker phase. The models make different predictions concerning the symmetry and history dependence of the circadian limits to FAA. To test those predictions, rats were entrained to 24-hr light–dark and feeding cycles and then exposed to feeding cycles of 21, 22, 25, 26, or 27 hr. Rats showed strong FAA to feeding cycles ≥ 24 hr, but not to schedules < 24 hr. Prior exposure to long cycles did not promote anticipation under short cycles. Meal omission tests confirmed that failure to observe FAA to < 24 -hr feeding cycles was not a result of masking by early mealtimes. These and other aspects of the results are consistent with the entrained oscillator model of FAA.

Food sources for many organisms may vary predictably in both place and time. For such organisms, optimal foraging may require anticipation of daily temporal windows of food availability. Anticipation of a time-limited feeding opportunity has been demonstrated in a few species studied in the field (see Aschoff, 1986; Boulos & Terman, 1980; Mistlberger, 1994). A more extensive literature has described food-anticipatory behavior in several common laboratory animals in much detail. This study is concerned with the mechanism by which one such species, the rat, is able to anticipate a scheduled daily meal.

Rats limited to a restricted amount or duration of food provided at a fixed time of day show, within a few days to a week, robust anticipatory behaviors, such as wheel running and operant lever responding, beginning 1–3 hr before mealtime. This food-anticipatory activity (FAA) shows a number of properties that distinguish it from anticipatory responding manifested under typical interval feeding schedules in the seconds-to-minutes range: (a) FAA emerges only if meal onset intervals are in the circadian range (approximately 22–31 hr; Stephan, 1981). (b) FAA may resynchronize to a shift of mealtime by a series of daily shifts, or transient cycles (Stephan, 1984). (c) FAA may exhibit free-running behavior if the feeding schedule is suddenly shortened from 24 to 22 hr (i.e., the daily rhythm of FAA persists but uncouples from mealtime and drifts with a stable but unique periodicity; Stephan, 1981, 1992). The daily rhythm of FAA also persists during prolonged food deprivation (Coleman, Harper, Clarke, & Armstrong,

1982; Rosenwasser, Pelchat, & Adler, 1984). These and other properties of FAA have been taken to suggest that FAA is regulated by a circadian clock or pacemaker that is entrained by circadian feeding schedules, analogous to entrainment of the daily rest–activity rhythm by 24-hr light–dark (LD) cycles. As ablations of the suprachiasmatic nuclei (SCN), which contain an identified circadian pacemaker, eliminate LD-entrained activity rhythms, but not FAA rhythms, the so-called food-entrainable pacemaker (FEP) is assumed to be distinct from the SCN-dependent, light-entrainable pacemaker (LEP; Boulos, Rosenwasser, & Terman, 1980; Stephan, Swann, & Sisk, 1979).

FAA has also been interpreted from a more traditional learning perspective as a form of conditioning, whereby circadian time serves as a discriminative or incentive stimulus for anticipatory responding. This interpretation has never been fully elaborated with respect to FAA and is either silent toward or inconsistent with many of the circadian properties of FAA (see Mistlberger, 1994). A more recent theoretical formulation has taken FAA as evidence that rats and other species store in memory a representation of circadian time (the momentary phase of a circadian pacemaker) that is linked with representations of the occurrence and place of feeding events (Gallistel, 1990). These representations can then be used in computations to predict future feeding times, and the predictions can be implemented by consulting the circadian pacemaker for current time. This representational–computational clock consultation model successfully accounts for some properties of FAA without recourse to entrainment of a separate circadian FEP but is weak in the face of other properties, including the appearance of free-running FAA cycles under 22-hr feeding cycles, the appearance and direction of transient cycles following meal shifts, and the persistence of FAA despite destruction of the SCN and loss of LD-entrained rhythms (reviewed in Mistlberger, 1994; see also Aschoff, 1986; Boulos & Terman, 1980).

The goal of this study was to assess anticipatory behavior in

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This research was supported by operating and equipment grants and a graduate scholarship from the Natural Sciences and Engineering Research Council of Canada.

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rats under feeding schedules designed to serve as additional tests of the entrained oscillator and computational learning models of FAA. Available evidence indicates that the circadian limits to anticipation are asymmetrical with respect to 24 hr. For example, SCN-ablated rats can anticipate meals separated by as much as 31 hr, but usually fail to anticipate meal onsets separated by less than 23 hr (Aschoff, von Goetz, & Honna, 1983; Stephan, 1981). Similarly, one preliminary study showed that intact rats entrained to a 24-hr LD cycle can anticipate a meal recurring at 25-hr intervals but not at 23-hr intervals (see Aschoff, 1986). The entrainment model can readily account for asymmetrical anticipation limits in terms of well-known principles from physical oscillator theory involving intrinsic pacemaker period (τ) and pacemaker resetting characteristics to the entraining stimulus. For example, the pacemaker could have an intrinsic $\tau > 24$ hr or could be capable of larger phase delays than phase advances in response to a daily feeding event (i.e., it may have an asymmetrical phase-response curve to resetting stimuli). These are not unreasonable characteristics for a circadian pacemaker (cf. Daan & Pittendrigh, 1976; Pittendrigh & Daan, 1976).

The computational model, by contrast, does not provide an obvious explanation for apparent asymmetries. According to this model, estimations of future feeding times are based on an average of the last 3–5 feeding times, with the most recent weighted preferentially in the computation. If mealtimes cluster sufficiently within a limited range of circadian phases (e.g., within a 4-hr window, as would occur on a 25-hr feeding cycle), the estimation will presumably be acted on. The probability level that is used for accepting a set of mealtimes as a meaningful cluster on which to base a prediction will determine whether an animal shows anticipatory behavior under a particular feeding cycle. The important point is that given a certain probability rule, it should be applied equally to feeding schedules longer and shorter than 24 hr. The circadian limits within which a rat can learn mealtimes should thus be symmetrical with respect to τ of the pacemaker. Associative learning models should make the same prediction.

Evidence for asymmetrical FAA limits might appear to constitute a strong case against computational and associative learning models of FAA. However, this evidence can be viewed as weak in two important respects. First, for arrhythmic, SCN-ablated rats, intrinsic τ of the FEP is unknown prior to restricted feeding, so it is not clear if the anticipation limits are truly asymmetrical with respect to τ . SCN-ablated rats anticipate a daily meal, and this FAA usually persists with a period of about 24 hr if the rat is food deprived. This suggests that following exposure to a 24-hr feeding cycle, τ of the clock is at least close to 24 hr. However, in SCN-ablated rats, FAA persistence is sometimes weak or variable in timing, thus compromising any assumption that $\tau = 24$ hr for a given case (Clarke & Coleman, 1986; Ruis, Talamini, Buijs, & Rietveld, 1989). By contrast, persistence of FAA during deprivation is both more robust and more temporally precise in intact rats that are adapted to 24-hr LD and 24-hr feeding schedules. The available evidence indicates that τ of the pacemaker driving FAA in LD-entrained intact rats is 24 hr (Coleman et al., 1982; Ottenwaller, Tapp, & Natelson, 1990; Rosenwasser et al., 1984). Therefore, observations of asymmetrical limits to FAA

in such rats should carry much weight in testing learning and entrainment models of FAA. However, there is only one preliminary study available (see Aschoff, 1986), and in that study only a limited range of feeding cycle durations and phase relations with the LD cycle were examined in an unspecified number of rats.

A second weakness in the evidence for asymmetrical FAA limits is that no procedures have been used to remove possible masking effects that might obscure evidence for synchronization of activity rhythms to <24 -hr feeding schedules. For example, a rat might learn or entrain to a 22-hr feeding cycle, but anticipatory activity might be absent because the computed estimate of feeding time or the phase-angle of entrainment is such that activity onset coincides with or occurs just after the actual mealtime (which begins 2 hr earlier each day). Expression of pacemaker-specified activity following mealtime might be masked by postprandial somnolence. Failure to observe anticipation to short feeding cycles can thus be ambiguous. However, this problem can be circumvented by meal omission tests, a procedure that was used in the present study.

An additional test of the entrainment and computational models follows from observations that the limits to FAA are influenced by the period (T) of prior feeding schedules. SCN-ablated rats can anticipate meals at intervals up to 31–33 hr, but only if T is gradually lengthened (Stephan, 1981). The entrainment model explains this as due to a change in τ of the FEP toward T of the entraining cycle. Such history dependence of τ is a well-known property of LEPs (Pittendrigh & Daan, 1976). The entrainment model explicitly predicts that as the upper limit to FAA (i.e., the limit to entrainment) is raised, the lower limit will also be raised; that is, it will be more difficult to entrain to a T cycle <24 hr if the rat was previously entrained to a T cycle $\gg 24$ hr. The computational model makes the opposite prediction. To anticipate meals under increasingly long T cycles, the probability rule for defining a significant cluster of mealtimes must be relaxed. If the rule is relaxed, this should apply equally to meal onset intervals less than 24 hr (i.e., as the upper limit to FAA is raised, the lower limit should be lowered to a similar degree). This study presents a test of these predictions.

General Method

A total of 48 male Wistar rats, weighing 250–300 g (Charles River, Quebec), were housed individually in Wahmann running wheel cages in light-controlled (LD 12:12), ventilated isolation cabinets. Each cage was equipped with an automated feeder that provided powdered rat chow through a window in the side cage door. Wheel revolutions were detected by microswitch closures monitored continuously by Apple computer. Activity counts were summed and stored at 5-min intervals and periodically downloaded to a Macintosh FX for analyses and display using Circadia (Behavioral Cybernetics, Cambridge, MA) and Systat (Systat, Evanston, IL). Student t tests (two-tailed) were used for statistical comparisons.

Experiment 1

One previous study, reported in preliminary form, showed that rats entrained to a 24-hr LD cycle can anticipate a daily meal recurring at 25-hr intervals (Aschoff, 1986). However, not

all possible phase relations between mealtime and the LD cycle were sampled or illustrated. A preliminary study was thus conducted to confirm and extend those findings.

Method

A total of 12 rats were subjected to the following sequence of feeding conditions: 2 weeks food ad libitum, no food for 24 hr, food 2 hr/day beginning 2 hr after lights-on for 22 days ($T = 24$ hr), food 2 hr beginning every 25 hr for 32 days ($T = 25$ hr).

Results and Discussion

All 12 rats showed robust FAA to mealtime under both the 24- and 25-hr feeding schedules (see Figure 1). FAA appeared within 2–7 days on the 24-hr cycle and ranged in duration from 2 to 4 hr across rats. Under the 25-hr schedule, FAA durations were on average longer and more variable within rats, as FAA moved in and out of phase with nocturnal activity. There was no phase of the LD cycle at which FAA was not expressed. The data thus confirm that intact, LD-entrained rats can be used to examine FAA under non-24-hr feeding cycles.

Two additional features of the data merit comment. During the first 1–2 weeks on the 24-hr feeding schedule, 7 of 12 rats exhibited a prominent band of activity that shifted from the dark period into the light period and toward the daily feeding time (see Figure 1A). One or more activity components remained nocturnal. Similar transients have been reported at least once previously and may be interpreted as reflecting the motion of a circadian oscillator in the process of entraining to feeding time (Persons, Stephan, & Bays, 1993). The transients suggest that prior to restricted feeding, the FEP, at least in some rats, resides in phase with the LEP. Computational and associative learning models provide no framework for interpreting the occurrence of transient cycles in the process of establishing FAA in an experimentally naive rat.

A second notable feature of the data is that, when the feeding schedule was lengthened from 24 to 25 hr, a component of FAA tended to persist at the original feeding time, resulting in a brief period of splitting, as a second FAA component tracked mealtime more closely (see Figure 1A and B). This lagging FAA component did not persist beyond the first 2 weeks on the 25-hr schedule. Analogous evidence for

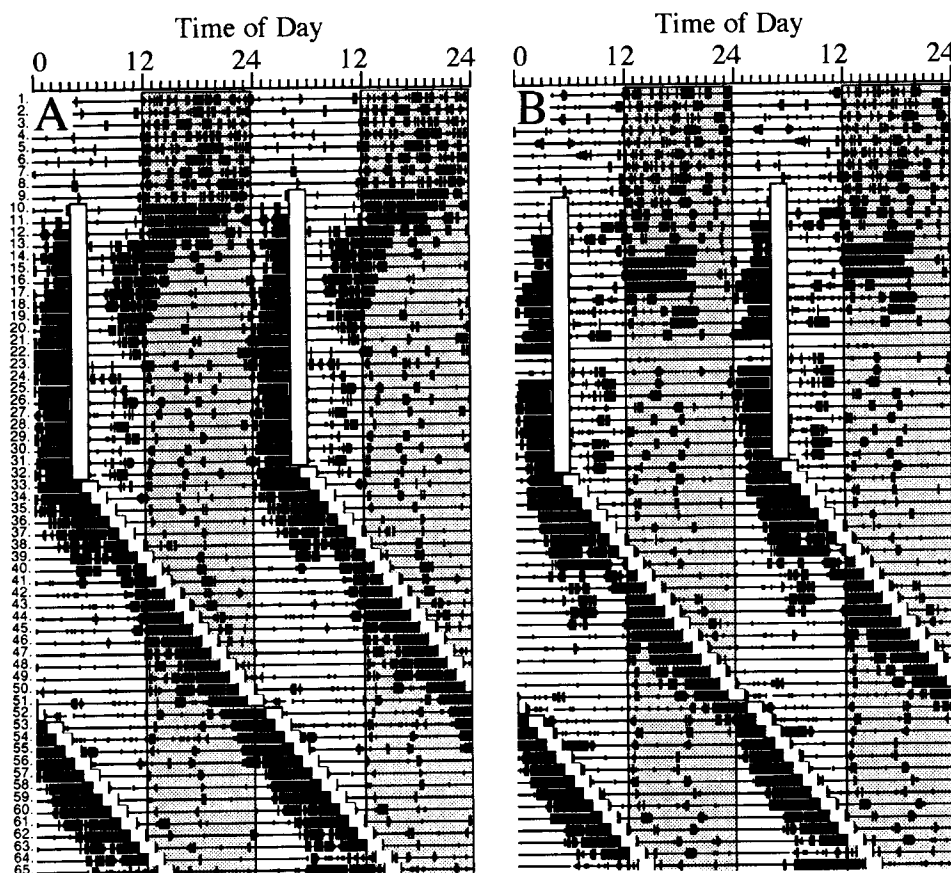


Figure 1. Wheel running activity charts from 2 rats subjected to 24- and 25-hr feeding cycles. The charts are double-plotted: each line presents 48 consecutive hr plotted in 10-min time bins from left to right. Consecutive days are also aligned vertically. Time bins during which activity occurred are denoted by vertical deflections proportionate to the amount of activity (three levels; 1–10 counts, 11–20 counts, >21 counts). Mealtime during food restriction is indicated by the vertical (24-hr feeding cycle) and oblique (25-hr feeding cycle) hollow bars. Lights-off is indicated by stippling.

persistence (or hysteresis) of some activity at a previous feeding time following reestablishment of FAA to a new mealtime has been observed previously in SCN-ablated rats (Stephan, 1992). The phenomenon has been interpreted as evidence that the FEP can dissociate into two components. The data presented here may be consistent with that model, although the mechanism for uncoupling two oscillators that are exposed to the same daily feeding event seems obscure. Alternatively, the apparent lagging of FAA onset may reflect a change in phase angle of two coupled FEPs as a transient response to the altered mealtiming. A third possibility is that FAA reflects two processes, a time-of-day association set up through consultation with the LD-entrained pacemaker and entrainment of a separate FEP. The time-of-day association would be expected to extinguish gradually after feeding time is shifted. The concept of two independent processes contributing to FAA will be considered further below. In any case, apparent, even transient splitting of FAA is not consistent with the predictions of the computational model; if FAA is an estimate of feeding time based on the previous few feeding times, with the last time weighted more heavily (Gallistel, 1990), then FAA should track a 25-hr meal smoothly without a prominent lagging component.

Experiment 2

In Experiment 2, restricted feeding schedules were lengthened from 24 to 26 hr in one set of rats and shortened from 24 to 22 hr in two additional sets of rats, as a first attempt to establish the symmetry of anticipation limits with respect to a 24-hr entrained circadian system. These schedules were selected on the basis of previous evidence that FAA in SCN-ablated rats is robust under 26-hr feeding cycles but absent under 22-hr cycles (Stephan, 1981). One possibility that has not been previously addressed is that animals might entrain to or learn a 22-hr cycle but with a lagging phase relation, so that anticipatory activity would tend to begin at or after mealtime rather than prior to mealtime. Such activity might then be masked by food presentation or postprandial somnolence, or by both. Thus, all of the rats were food deprived for at least 72 hr following the 26- and 22-hr schedules. If animals have learned something about mealtime, either by entrainment, computations, or associations, this should be evident when meals are omitted for one or more cycles.

Method

A total of 6 rats were fed ad libitum for 12 days and were then restricted to 3 hr food/day beginning 4 hr after lights-on for 16 days. Mealtime was then delayed by 1 hr/day for 3 days ($T = 25$ hr) and then by 2 hr/day for 24 days ($T = 26$ hr). Food was removed for 80 hr, provided ad libitum for 5 days, and then removed again for 70 hr. The results suggested that further testing might provide useful interpretive information; thus, all of the rats were subjected to a second and third 24-hr feeding schedule, followed by multiple 70–90-hr bouts of total food deprivation. The sequence of conditions are illustrated in Figure 2 and will be elaborated in the Results and Discussion section.

A second group of 6 rats were fed ad libitum for 2 weeks and then restricted to 3 hr food/day beginning 4 hr after lights-on for 17 days. Mealtime was then advanced by 1 hr/day for 3 days ($T = 23$ hr) and

then by 2 hr/day for 21 days ($T = 22$ hr). Meal duration was shortened to 90 min/day to compensate for the 4-hr difference in meal onset intervals between the 22- and 26-hr schedules (but note that FAA is remarkably similar across a wide range of meal durations, thus meal duration should not be a significant factor; cf. Mistlberger, 1994; Stephan & Becker, 1989). The rats were then food deprived for 80 hr, fed ad libitum for 5 days, and food deprived again for 72 hr.

A third group of 12 rats received food ad libitum for 2 weeks and were then restricted to 3 hr food/day beginning 4 hr after dark onset for 25 days. Food was then advanced by 1 hr/day for 3 days ($T = 23$ hr) and then by 2 hr/day for 14 days ($T = 22$ hr), concomitant with a reduction of meal duration to 90 min. Food was then removed for 60 hr, provided ad libitum for 3 days, and removed again for 48 hr.

To estimate τ of FAA bouts during 3–4 cycles of food deprivation, the onset of FAA bouts was defined by computer algorithm as the first of two consecutive 10-min bins in the lights-on period during which activity exceeded the 10-min mean for the day.

Results and Discussion

All six rats exhibited robust FAA under the 24-hr and 26-hr feeding schedules (see Figure 2A and B). When food was removed for 80 hr, the daily rhythm of FAA persisted strongly, with little evidence of damping over three complete cycles.

None of the 18 rats tested under the 22-hr feeding schedule showed evidence of FAA (see Figure 3A, B, and C). The 6 rats that were fed in the light period during the initial 24-hr schedule showed, in most cases, complex disruptions of activity (see Figure 3A), with bands of activity persisting in the light period without clear coupling to the advancing mealtime. When food was removed for a 72-hr period in these rats, a great deal of activity appeared in the light period, particularly around the last scheduled feeding time (see Figure 3A). However, this also corresponded to the approximate feeding time under the initial 24-hr schedule; activity may have persisted at that original circadian phase (cf. Coleman et al., 1982; Rosenwasser et al., 1984).

The other 12 rats subjected to 22-hr feeding cycles were intended to control for that possibility. These rats were initially entrained to a 3-hr meal in the dark period and were food deprived after the 14th meal of the 22-hr schedule, which ended in the lights-on period. These rats showed no complex disruptions of activity, although there were modulations of nocturnal activity that, in some cases, resembled relative coordination to the daily advancing mealtime (see Figure 3B and C). FAA did not track mealtime into the light period in any animal.

These results would appear to establish that the circadian limits to FAA are asymmetrical with respect to 24 hr; FAA faithfully tracks a mealtime that delays by 2 hr/day but does not track a mealtime that advances by 2 hr/day. However, when the rats were food deprived following the 22-hr schedule, many did exhibit some diurnal activity beginning at or just after the time of day that they last received food (see Figure 3C). The deprivation results thus appear to argue against asymmetrical limits, at least in the 22–26-hr range. Nonetheless, 3 of 11 rats showed little or no evidence for diurnal activity during deprivation (see Figure 3B). In addition, the amount of diurnal activity expressed during the deprivation test was in all of the rats far less than was displayed by rats during deprivations that followed the 26-hr feeding schedule (LD ratios averaged over

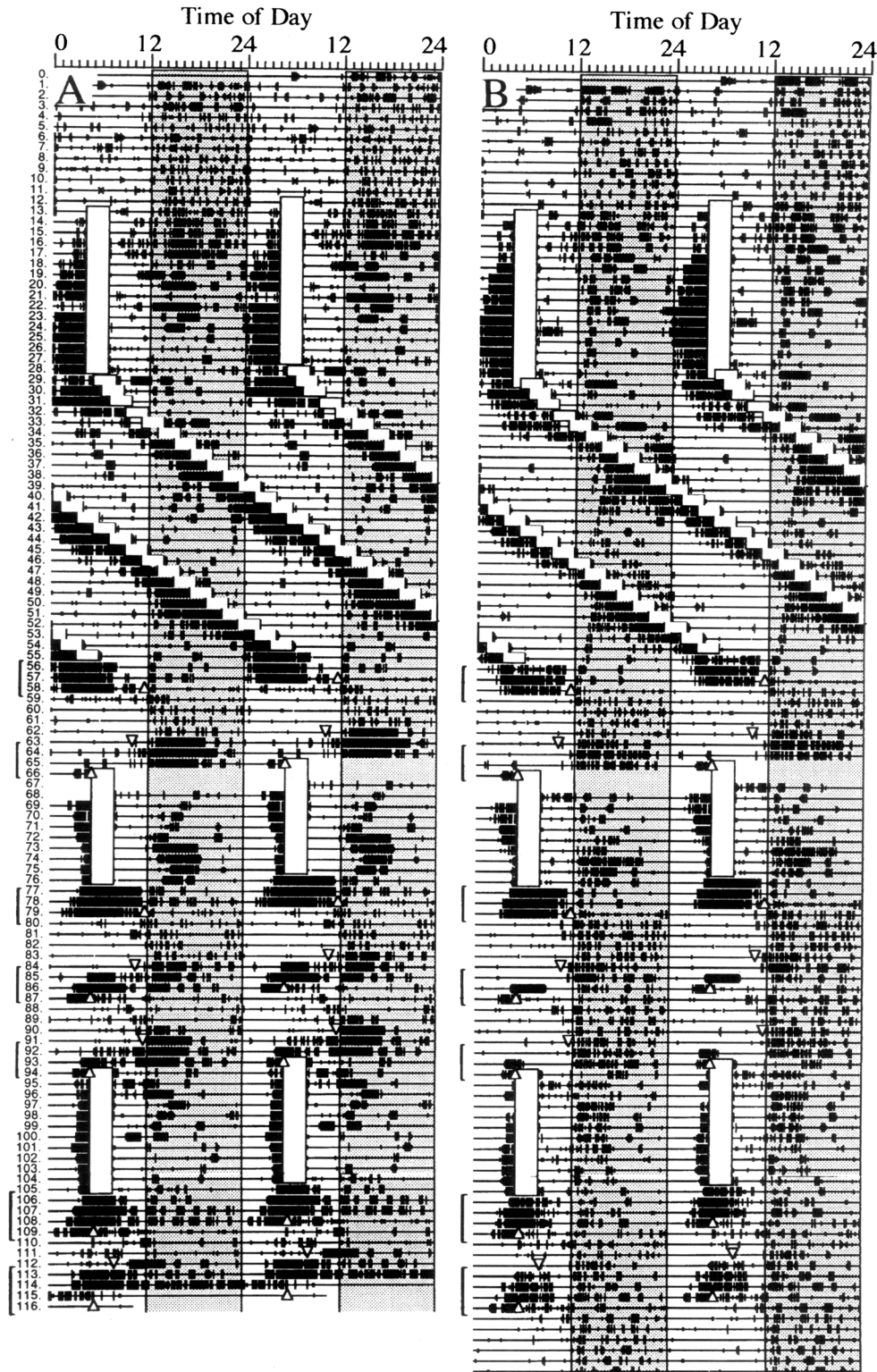


Figure 2. Double-plotted wheel-running charts from 2 rats subjected to 24-hr and 26-hr feeding schedules. Each line presents 48 consecutive hr plotted in 10-min time bins from left to right. Consecutive days are also aligned vertically. Time bins during which activity occurred are denoted by vertical deflections proportionate to the amount of activity (three levels; 1-10 counts, 11-20 counts, > 21 counts). Mealtime during food restriction is indicated by the vertical (24-hr feeding cycle) and oblique (26-hr feeding cycle) hollow bars. Lights-off is indicated by stippling. Food-deprivation days are indicated by vertical bars to the left of each chart. Triangles and inverted triangles denote the end and beginning, respectively, of food deprivation episodes. Blanks indicate data missing because of equipment failure.

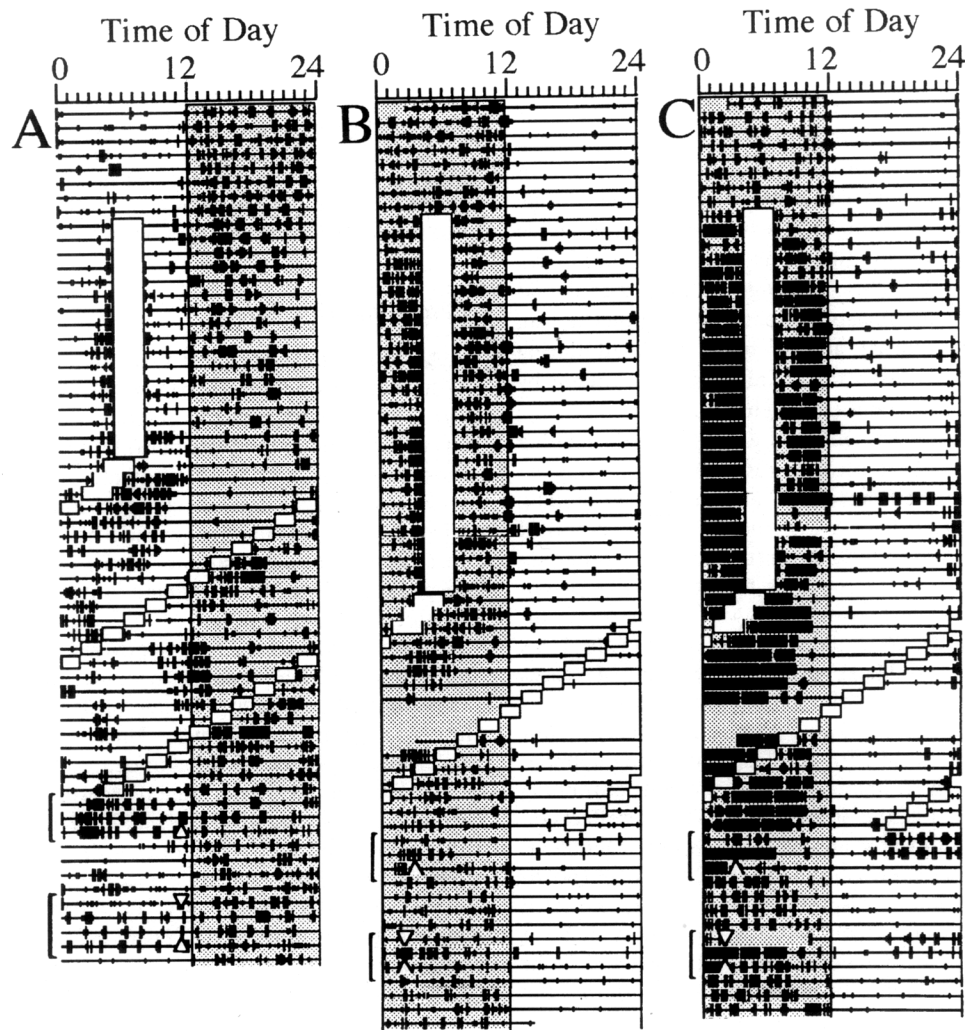


Figure 3. Single-plotted wheel-running charts from 3 rats subjected to 24-hr and 22-hr feeding cycles. Each line presents 24 consecutive hr plotted in 10-min time bins from left to right. Consecutive days are also aligned vertically. Time bins during which activity occurred are denoted by vertical deflections proportionate to the amount of activity (three levels; 1–10 counts, 11–20 counts, >21 counts). Mealtime during food restriction is indicated by the vertical (24-hr feeding cycle) and oblique (22-hr feeding cycle) hollow bars. Lights-off is indicated by stippling. Food-deprivation days are indicated by vertical bars to the left of each chart. Triangles and inverted triangles denote the end and beginning, respectively, of food-deprivation episodes. A: Rat subjected to 24-hr feeding schedule with 3 hr mealtime in the lights-on period. B and C: Two rats subjected to 24-hr feeding schedules with mealtime in the lights-off period.

the 2nd and 3rd days of food deprivation were significantly different between the 22-hr and 26-hr T-cycle groups, $t = 2.9$, $p = .011$; LD ratios during ad-lib food days did not differ between groups, $t = -0.51$, $p = .57$). These results thus indicate that 22-hr schedules, but not 26-hr schedules, do fall below the lower circadian limits of FAA in some cases and at least approach these limits in others.

During the food deprivation that immediately followed the 26-hr schedule, all 6 rats showed very strong persistence of FAA in the lights-on period, when the last meal occurred. However, during a second food-deprivation test 5 days later, surprisingly little diurnal activity was evident. Instead, noctur-

nal activity levels were elevated, particularly early in the dark period (see Figure 2A and B). This corresponded to the time that FAA would have appeared, had the 26-hr feeding schedule been maintained. Previous studies in this and other laboratories have shown that during food-deprivation tests imposed within a week of a 24-hr diurnal feeding schedule, diurnal activity is greatly increased and nocturnal activity usually decreased (e.g., Clarke & Coleman, 1986; Coleman et al., 1982; Rosenwasser et al., 1984).

One possible explanation for this result is that the refeeding event after the first 3-day deprivation period, which occurred at dark onset, caused FAA to reset to that time. To test this

hypothesis, the 6 rats were subjected to a second 24-hr feeding schedule for 12 days, were food deprived for 80 hr, and were then refeed at dark onset. After 5 days, food was again removed for 60 hr. LD ratios were then calculated for the first 2 deprivation days of this second deprivation test and compared with the second deprivation test that followed the 26-hr schedule. Diurnal activity was significantly greater during deprivations that followed the 24-hr schedule (LD ratios were $.19 \pm .10$ and $.87 \pm .69$ for the 26-hr and 24-hr conditions, respectively; $t = 2.6$, $p = .04$). This suggests that refeeding at dark onset does not appreciably reset the timing mechanism that generates the diurnal activity bouts revealed during subsequent food-deprivation tests. This is clearly evident by visual inspection of actograms (see Figure 2).

All of the rats were next subjected to a third 24-hr feeding schedule and two more deprivation tests. Over the 3 days of deprivation that followed the second and third 24-hr feeding schedules, the onset of the FAA rhythm advanced (occurred earlier; see Figure 2) in 11 of 11 cases (two tests/rat, with data from 1 rat during one test missing because of equipment failure). The mean advance over 3 days was $1.41 \text{ hr} \pm 0.25 \text{ hr}$. However, following the 26-hr schedule, the onset of the FAA rhythm delayed (occurred later) over the 3 days in 5 of 6 cases ($1.01 \text{ hr} \pm 1.63 \text{ hr}$). Together with the LD ratio results, this can be interpreted within the framework of the entrained oscillator model as evidence that an FEP can free-run during deprivation, with a τ that approximates T of the previous entraining feeding schedule; that is, the oscillator exhibits aftereffects on τ plus momentum, and, in the absence of a feeding schedule, is not necessarily immediately coupled to the 24-hr LEP (possibly because of the forced disparity between FEP and LEP periodicities). This would account for the progressive delay of FAA onset during the first deprivation following the 26-hr schedule and the prevalence of nocturnal activity during the second deprivation. Conditioning and computational learning models do not offer a ready interpretation for these results.

The concept of an FEP exhibiting momentum does not explain the small amount of diurnal activity that did appear in 5 of 6 rats during the second deprivation test that followed the 26-hr feeding schedule. This may indicate that two timing mechanisms are in operation; an FEP that is independent of the SCN-LEP and a second time-marking system (associations or computations) that uses phase information provided by the SCN-LEP, which appears to maintain a relatively stable phase during and after the feeding schedule. Thus, during deprivation, activity may conceivably appear at different times, specified by different mechanisms, although in the common case in which LEP and FEP periods are identical (as under concurrent 24-hr feeding and LD schedules), such dissociations would not be evident.

Experiment 3

Experiment 2 provided evidence for asymmetric limits to FAA in separate groups of rats. In Experiment 3, the symmetry of FAA limits was assessed using a within-subjects design. This provided an additional opportunity to test specific predictions concerning the history dependence of FAA.

Method

A total of 12 rats received food ad libitum for 2 weeks and were then restricted to food for 3 hr/day beginning 5 hr after dark onset for 16 days. Mealtime was delayed by 1 hr/day for 2 days ($T = 25 \text{ hr}$), by 2 hr/day for 8 days ($T = 26 \text{ hr}$), and by 3 hr/day for 12 days ($T = 27 \text{ hr}$). Feeding duration was then reduced to 90 min and advanced by 3 hr/day for the next 14 days ($T = 21 \text{ hr}$). Food was then removed for 80 hr, provided ad libitum for 5 days, and removed again for 72 hr.

Results and Discussion

All 12 rats showed robust FAA under the 24-, 25-, 26-, and 27-hr feeding schedules, but in no case did FAA established to the 27-hr schedule reverse direction and track mealtime as it advanced through the light period during the 21-hr schedule. Most rats instead showed modulations of nocturnal activity suggestive of weak relative coordination to the 21-hr feeding schedule. The results thus provide no evidence that rats can entrain to or predict mealtimes occurring 3 hr earlier each day, despite exhibiting robust anticipation of mealtimes occurring 3 hr later each day.

However, when food was removed for 3 days immediately following the 21-hr feeding schedule, many rats did exhibit some diurnal activity at the time of day corresponding to the last one or two mealtimes. Thus, as in the $T = 22\text{-hr}$ experiment, food deprivation appeared to reveal evidence for a memory of mealtime. Again, however, several rats that exhibited consistent FAA to the 27-hr feeding cycle showed little or no diurnal activity during the food deprivation that followed the 21-hr schedule (see Figure 4B). In addition, several rats (see Figure 4A) exhibited transient bouts of diurnal activity during the 21-hr cycle, which suggests the following interpretation: The 21-hr schedule, and possibly the 22-hr schedule used in Experiment 2, may transiently alter the phase of the LEP, or may induce relative coordination of a FEP, causing it to advance partially into the light period. The diurnal activity evident during the deprivation test may thus simply represent a partially shifted FEP or LEP and not a specific memory for mealtime based on true entrainment, conditioning, or computations. In other studies (Mistlberger, 1992), we have observed that a single 2-hr meal provided early in the light period following 48 hr of food deprivation is associated with increased activity during the last 4 hr (but not the first 8 hr) of the light period on 2 subsequent days of food deprivation. This suggests that even a single diurnal feeding event in a hungry rat may phase advance the LEP, although it is evidently not sufficient to produce a memory for the feeding time. Increased activity late in the day in food-deprived rats thus need not represent FEP or computational processes.

General Discussion

The following results reported here are readily interpreted by the entrained oscillator model of FAA. These include, (a) transient cycles of nocturnal activity moving toward mealtime during the first 2 weeks of a diurnal 24-hr feeding schedule, (b) asymmetrical limits to FAA in the 22–26-hr range, (c) failure of prior exposure to a 27-hr feeding cycle to promote anticipa-

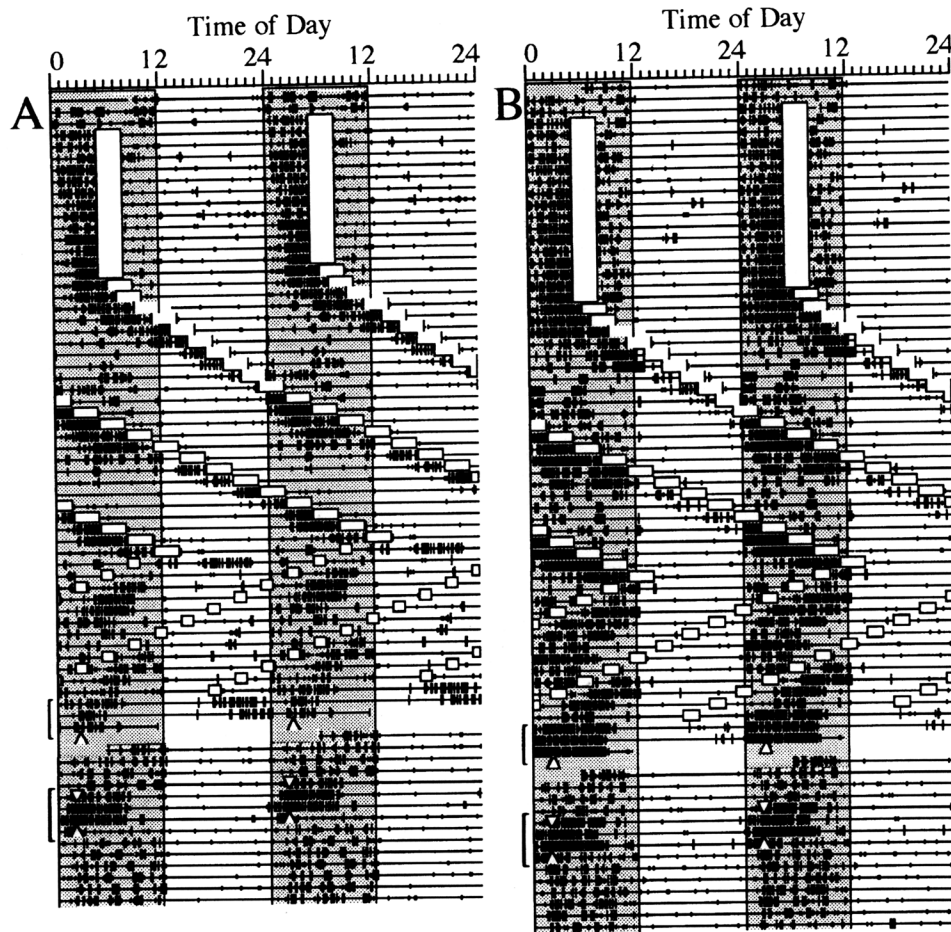


Figure 4. Double-plotted wheel-running charts from 2 rats subjected to 24-, 25-, 26-, 27- and 21-hr feeding cycles. Each line presents 48 consecutive hr plotted in 10-min time bins from left to right. Consecutive days are also aligned vertically. Time bins during which activity occurred are denoted by vertical deflections proportionate to the amount of activity (three levels; 1–10 counts, 11–20 counts, > 21 counts). Mealtime during food restriction is indicated by the vertical (24-hr feeding cycle) and oblique (non-24-hr feeding cycles) hollow bars. Lights-off is indicated by stippling. Food-deprivation days are indicated by vertical bars to the left of each chart. Triangles and inverted triangles denote the end and beginning, respectively, of food-deprivation episodes.

tion or coordination of activity to a 21-hr schedule, and (d) possible τ aftereffects and momentum of FAA, with $\tau > 24$ revealed during food-deprivation tests that followed a 26-hr feeding cycle.

A clock consultation–computational model does not speak to the phenomenon of transients in experimentally naive rats, nor is it easily compatible with evidence for asymmetrical anticipation limits with respect to 24 hr. Asymmetrical limits can be accommodated only if it is assumed that the consulted clock is not the circadian pacemaker that is entrained to the 24-hr LD cycle but is some other self-sustaining circadian oscillator that has a $\tau > 24$ hr. If this oscillator is not entrained by either light or food, then its τ and phase would remain unknown, and one could not assume (without resorting to coincidence) that these clock parameters would be identical (i.e., synchronized) across animals. Consequently, this type of free-floating oscillator model would not explain the apparent

momentum of FAA observed after the 26-hr feeding schedule, or the timing of diurnal activity following the 24-hr feeding schedules, as the activity patterns evident during the food-deprivation tests were closely synchronized across rats. Such group synchrony would be expected only if phase and period of the consulted clock were controlled (i.e., entrained) by the restricted feeding cycles to which all of the rats were previously exposed.

The weight of evidence from this behavioral study of intact rats, and from previous ablation studies, indicates that restricted daily feeding schedules entrain a circadian pacemaker located outside of the SCN and that this mechanism is sufficient to explain anticipation of a single daily meal. If circadian phase can be encoded in memory on the basis of clock consultation, it would seem likely that the consulted clock is also light or food entrainable. The present study provides no strong evidence for such consultation and coding

processes, although the appearance of diurnal activity during the second deprivation that followed the 26-hr schedule (Experiment 2) could be interpreted in terms of a second timing mechanism, if the FEP momentum hypothesis is accepted.

For animals to adapt optimally to more complex feeding schedules in which two or more feeding times are linked to unique feeding places, it may be required that both time and place be encoded memorially. Time-place learning under such schedules has been observed in several species (Biebach, Falk, & Krebs, 1991; Boulos & Logothetis, 1990; Daan, Leiwakabessy, Overkamp, & Gerkema, 1994), but whether it is a LEP or FEP that serves as the consulted clock has not been clearly established. SCN-ablated rats can anticipate two (but not three) daily meals found in the same place, and there is limited evidence that they can acquire a two meal time-place association, suggesting that the FEP can be consulted (Boulos & Logothetis, 1990; Mistlberger, Bossert, DeGroot, & Marchant, 1995). Definitive evaluation of the role of the SCN-LEP as a consulted clock may require removal of the FEP, the location of which is not presently known.

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Received August 15, 1994

Revision received February 1, 1995

Accepted February 1, 1995 ■