Limits of maternal entrainment of the activity rhythm in the field mouse *Mus booduga*

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Summary. Cycles of 12 h presence and 12 h absence of mother *Mus booduga* entrain the circadian rhythm in the locomotor activity of her pups such that the pups rest in her presence and are active in her absence. We wanted to determine whether this maternal entrainment arises because activity is inhibited by the mother's presence and enhanced by her absence (masking). We performed experiments with the period of the presence/absence cycles ranging from 20 to 28 h and find that only periods of 23–25 h allow entrainment while periods below 23 h and above 25 h do not allow entrainment. Our results speak against the involvement of masking and in favour of the involvement of a genuine circadian organization.

Introduction

In infants of the field mouse *Mus booduga*, the presence and absence (PA) cycles of the mother entrain the pups' circadian locomotory activity rhythm in constant conditions such that the pups rest in the mother's presence and are active in her absence (Viswanathan and Chandrashekaran 1985a, 1987; Viswanathan 1987). In those experiments the mother was physically presented for 12 h and removed for 12 h (a period, T, of 24 h). Clearly, these PA cycles act as a zeitgeber. A zeitgeber may affect a circadian rhythm in at least two different ways: (a) it may entrain the rhythm via circadian transducing mechanisms; and (b) it may influence (mask) the overt rhythm more directly without involving the circadian organization (Enright 1965; Hoffmann 1969; Aschoff et al. 1982).

Abbreviations: PA presence and absence of mother; *LD* light and darkness; *DD* continuous darkness; *T* period of zeitgeber; *τ* period of activity rhythm; *ψ* phase angle difference

Separation of the mother from infants in guinea pigs, monkeys and rats results in an increase in sleep disturbance and possibly enhanced activity (Astic and Jouvet-Mounier 1968; Reite and Short 1978; Hofer and Shair 1982). It is thus also possible that the entrainment of the activity rhythm of pups to PA cycles reported in our earlier studies was not genuine and may have been just a result of masking, i.e. of direct imposition of rest and release of activity by the mother's presence and absence respectively. One way to test whether activity and rest in the pups are directly caused by the absence and presence of the mother is to create PA cycles, the T of which vary markedly from 24 h in both directions. If masking were involved then the rhythm would be entrained to an imposed cycles of any T and result in a wide range of periods of entrainment. Conversely, if entrainment is by means of circadian mechanisms the range of T would be appropriately narrow or limited. We therefore performed a series of experiments in which the T of the maternal PA cycles imposed on *Mus booduga* pups ranged from 20 to 28 h and report here that cycles with a T of 23–25 h successfully entrain the rhythm while cycles with a T below 23 h or above 25 h fail to entrain. The limits of maternal entrainment thus lie between 23 and 25 h, implicating circadian transduction.

Materials and methods

Pregnant *Mus booduga* were captured from the fields surrounding the University campus, maintained in continuous darkness (DD) and divided into batches of four to six animals. They littered two to eight pups each. Two pups, one of each sex, were selected from a litter and placed in plastic boxes of $21 \times 15 \times 13$ cm. Starting on day 5 of the pups' lives, mothers were alternately presented and removed for the various batches for 10, 11, 11.5, 12, 12.5, 13 and 14 h, thereby creating PA

cycles of 10:10 h (T=20 h), 11:11 h (T=22 h), 11.5:11.5 h (T=23 h), 12:12 h (T=24 h), 12.5:12.5 h (T=25 h), 13:13 h (T=26 h) and 14:14 h (T=28 h). Thus, each of the two pups of every batch would be with the mother for exactly one-half of the cycle. On day 16 the pups were introduced into running wheels and their locomotor activity was recorded using a A 620 × Esterline Angus Event Recorder. A continuous record of the time of activity was made on the recorder, which moved the paper roll at a uniform rate of 30 cm/day. The PA cycles were continued until between days 23 and 30, but the mother was tethered by an aluminium chain barring her from entering the wheel. The mother/infant interactions thus took place in the nesting cage attached to the wheels. Food (millet, maize and other grain) and water were supplied ad libitum. In DD, red light of >610 nm was used for a short duration as a 'safe light' for cleaning the cages and wheels, and for transfer of animals etc. (Viswanathan and Chandrashekaran 1985b).

During entrainment to cycles with Ts of 23, 24 and 25 h, the phase relationship between onset of a pup's activity and the time of removal of the mother was determined and averaged for each animal.

The estimation of the freerunning period (τ) in DD was always based on the onset of activity, which, in the majority of records, displays greater precision than the midpoint or the end of activity (Enright 1965; Chandrashekaran et al. 1983).

Data shown in Table 2 were subjected to non-parametric statistical analyses using the Spearman rank correlation and the Mann-Whitney test (Zar 1984) and the Spearman rank correlation was used with the data shown in Table 3.

Results

The effect of PA cycles of different T on the circadian activity rhythm of pups is indicated in Ta-

Table 1. Effect of PA cycles of different periods (T) on the circadian rhythm of pups

	Zeitgeber period (h)						
	20	22	23	24	25	26	28
Number entrained/ number tested	0/4	0/5	5/5	10/10	6/6	0/4	0/6
Percent (%) entrainment	0	0	100	100	100	0	0

ble 1. The PA cycles of 11.5:11.5 h (Fig. 1a), 12:12 h (Fig. 1b) and 12.5:12.5 h (Fig. 1c) successfully entrained the activity rhythms of pups in DD. The activity generally began immediately after the removal of mother but in some cases the following mean phase angle differences (ψ) were found: during entrainment with a T of 23 h, three out of five animals showed a ψ of 0.12 h; with a T of 24 h, two out of ten animals showed a ψ of −0.09 h; with a T of 25 h, three out of six animals showed a ψ of 0.65 h.

The PA cycles with a T of 23, 24 and 25 h were discontinued between days 23 and 30. The τ of the freerunning rhythm in the subsequent DD reflects the influence of the T of the previous entraining PA cycles. Such effects on the τ of the animals as were found after the discontinuation are indicated in Table 2. There is a positive correlation between the τ of the freerunning rhythm and the T of the PA cycles until days 51–60. For instance, in those animals which experienced PA cycles with a T of 25 h, the mean value of τ (days 31–40) was 24.36 h, while in those that experienced PA cycles with a T of 23 h, the mean value of τ was 23.23 h. These values are larger and smaller, respectively, than the mean value of τ (23.68 h) for animals which experienced PA cycles with a T of 24 h (Table 2). Comparing the freerunning periods between T=23 h and T=24 h animals and between T=24 h and T=25 h animals shows that there are clear-cut differences (Table 2). However, such differences disappear after day 50.

The locomotor activity rhythm of the pups failed to entrain to the rather markedly deviant PA cycles of 10:10 h (Fig. 2a), 11:11 h (Fig. 2b), 13:13 h (Fig. 2c) and 14:14 h (Fig. 2d): the activity rhythm of all the pups freeran. The freerunning period of activity and changes in it are shown for every ten days in Table 3. No correlation between τ and T was found either during or after the PA cycles (Table 3; P>0.05).
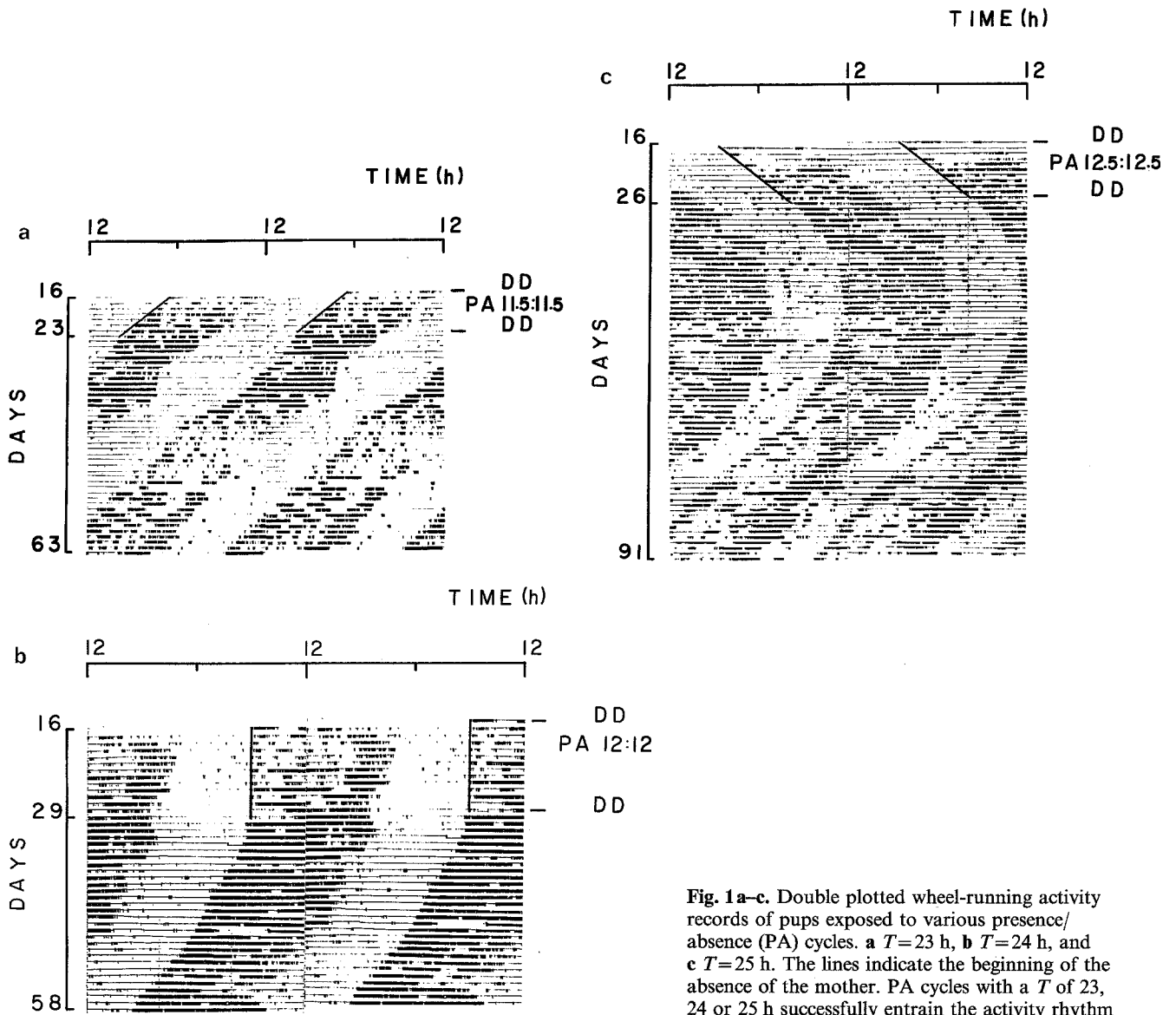
Table 2. Aftereffects of entraining to PA cycles of different periods (T)

Days	T=23 h		T=24 h		T=25 h		Spearman rank correlation coefficient (r_s) of T and τ	Values of Mann-Whitney test (U)	
	n	$\bar{\tau}\pm$SD	n	$\bar{\tau}\pm$SD	n	$\bar{\tau}\pm$SD		τ of T=23 and τ of T=24	τ of T=24 and τ of T=25
31–40	5	23.23±0.44	10	23.68±0.22	6	24.36±0.47	0.716 (P<0.001)	41.5 (P<0.05)	52 (P=0.01)
41–50	4	23.31±0.24	10	23.46±0.23	6	23.88±0.26	0.658 (P<0.005)	36 (P<0.025)	53.5 (P<0.01)
51–60	4	23.25±0.21	8	23.28±0.34	6	23.75±0.50	0.550 (P=0.02)	17[a]	37.5[a]
61–70	3	23.17±0.22	7	23.20±0.51	6	23.74±0.53	0.495[a]	11[a]	30[a]
71–80	3	23.10±0.30	4	23.28±0.54	6	23.65±0.59	0.469[a]	8[a]	15[a]

[a] Not significant at 5% probability level

Fig. 1a–c. Double plotted wheel-running activity records of pups exposed to various presence/ absence (PA) cycles. **a** $T=23$ h, **b** $T=24$ h, and **c** $T=25$ h. The lines indicate the beginning of the absence of the mother. PA cycles with a T of 23, 24 or 25 h successfully entrain the activity rhythm

Table 3. Mean period ($\bar{\tau}$) during and after PA cycles of different periods (T)

Days	$T=20$ h		$T=22$ h		$T=26$ h		$T=28$ h		Spearman rank correlation coefficient (r_s) of T and τ
	n	$\bar{\tau} \pm$ SD	n	$\bar{\tau} \pm$ SD	n	$\bar{\tau} \pm$ SD	n	$\bar{\tau} \pm$ SD	
21–30[a]	3	23.55±0.27	5	23.65±0.65	4	24.15±0.13	5	23.84±0.21	0.314[b]
31–40[a]	2	23.71	5	23.65±0.42	4	24.14±0.26	6	23.92±0.19	0.355[b]
41–50[a]	1	23.62	4	23.52±0.50	1	24.17	6	23.80±0.26	0.291[b]
51–60	4	23.53±0.31	4	23.35±0.39	3	23.90±0.30	6	23.72±0.22	0.383[b]
61–70	4	23.38±0.48	4	23.38±0.52	2	23.87	6	23.79±0.15	0.471[b]
71–80	2	23.39	3	23.04±0.71	2	23.90	6	23.66±0.29	0.470[b]

[a] Days during PA cycles
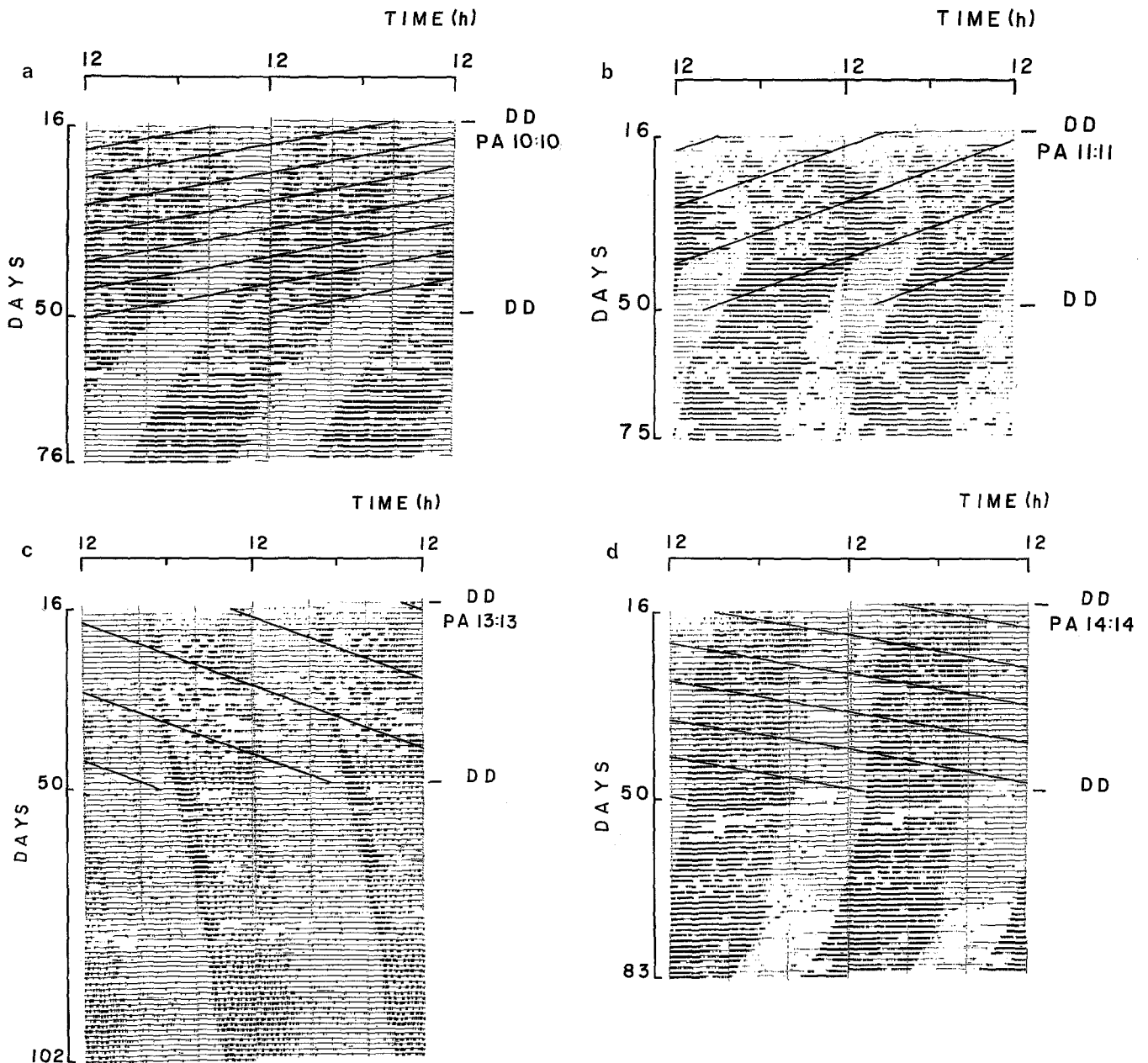[b] Not significant at 5% probability level

Fig. 2. Double plotted wheel-running activity records of pups exposed to various presence/absence (PA) cycles. **a** $T=20$ h, **b** $T=22$ h, **c** $T=26$ h, and **d** $T=28$ h. The lines indicate the beginning of the absence of the mother. PA cycles were discontinued in all cases on day 50, indicated by the ending of the lines. Cycles of these Ts failed to entrain the activity rhythm

Discussion

The results of the present investigation clearly show that the PA cycles act as genuine zeitgebers in entraining the locomotor activity rhythm of pups and the circadian timing mechanisms which underlie it. The activity rhythm synchronized to PA cycles only when the imposed T did not deviate greatly from 24 h ($T=23$–25 h; Fig. 1). Such a limited range of entrainment and the capacity to time

the onset of freerunning activity by the zeitgeber (PA cycles) clearly indicate that the rhythm reported here is circadian.

An entrained rhythm is expected to change its phase relationship to the zeitgeber in a systematic manner, positive phase angle differences becoming larger as T increases (Pittendrigh and Daan 1976; Aschoff 1978; Aschoff and Pohl 1978). This occurred in our study, although the ψ values were estimated only for some animals. The ψ value for

PA cycles with a T of 25 h is more positive (0.65 h; $n=3$) than that for PA cycles with a T of 23 h (0.12 h; $n=3$).

The T of the PA cycles influences the τ of the freerunning rhythm in the subsequent DD. These differences in τ observed after discontinuation of the PA cycles may well be an aftereffect (Table 2). This suggests that the PA cycles not only entrain the circadian activity rhythm to their own period but can also have a long-lasting effect on the period of the pups. The extent to which non-24-h cycles can change the period of the pacemaker and the rate at which such changes decay under constant conditions, both measures of pacemaker 'plasticity', have been extensively studied in adult animals (Pittendrigh 1960; Pittendrigh and Daan 1976). It has been found that aftereffects in adult mice last for up to 100 days in DD. However, the results in our younger animals (Table 2) clearly show that the induced changes (aftereffects) in τ following the PA cycles are not permanent and last only for a period of 3–5 weeks. This relatively rapid decay of aftereffects in our mice may be due to the age of the animals.

Similar temporary aftereffects lasting approximately 25 days have been reported for the circadian rhythms of young mice following LD cycles with a T of 20 h or 28 h (Davis and Menaker 1981). The results of Davis and Menaker and those of the present study indicate that the adult circadian pacemaker is affected by the entraining cycle to a greater extent than that of the developing animal. The pacemaker plasticity is greater in young mice; changes occur more rapidly in young than in old mice.

No entrainment ensues if the T is less than 23 h or more than 25 h and the activity rhythms freerun when the T of the PA cycles is outside this range (Fig. 2). Furthermore, these cycles failed to influence the τ of the activity rhythm, nor could any aftereffects be found (Table 3). These results provide further evidence that entrainment of activity ensues through a genuine circadian organization and is not brought about by masking effects.

It has been reported that anticipatory activity could be induced in the freerunning rhythm of intact rats or of rats with suprachiasmatic nuclei (SCN) lesions only if the T of the feeding schedule was between 22 and 31 h (Boulos et al. 1980; Stephan 1982). Tribukait (1956) studied the rhythmic behaviour of mice entrained to various Ts (equal periods of light and dark). He found that the activity rhythms could be entrained if T was 21–28 h and that they failed to synchronize if T was longer or shorter than these limits. Similar results have

also been reported in other animal systems (Pittendrigh and Daan 1976; Aschoff 1978; Moore-Ede et al. 1982). Furthermore, it has been found that, in mice raised under non-environmental cycles of various periods, activity was also entrained to 20-h and 28-h LD cycles. The limits of entrainment to LD cycles have not been worked out for *Mus booduga*, but based on the reports of other authors and on the present study it appears that the range of entrainment for a particular rhythm may differ from one zeitgeber to another. The range of entrainment may even differ between various rhythms of the same system (Simpson et al. 1970; Aschoff and Wever 1976; Aschoff 1978; Sulzman et al. 1978). Further studies of *Mus booduga* pups with maternal PA cycles need to be undertaken to see to what extent the locomotory activity may be representative of the range and limits of entrainment for the whole circadian system.

In fact, it is now believed that activity associated with anticipation of food and entrained to feeding/starvation cycles is mediated by a circadian mechanism which is functionally independent of the SCN. This subsidiary circadian mechanism often shows several similarities to the circadian rhythms entrained by LD cycles, but there are also several differences between the two circadian systems (Rosenwasser et al. 1984; Stephan 1984). Similarly, perch-hopping and feeding rhythms have been postulated to be governed by two or more oscillators in passerine birds (Subbaraj and Gwinner 1985; Gwinner et al. 1987). The entraining of the pups' locomotor activity rhythm to the PA cycles of the mother in *Mus booduga* may represent the principal circadian oscillation, which itself yields to LD entrainment later in development (N. Viswanathan, unpublished). The actual factor behind maternal entrainment (what is it in the mother that entrains?) has also to be determined.

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