

# Role of relative durations of presence/absence of mother mouse (*Mus booduga*) in circadian rhythm of pups

N. Viswanathan

Department of Animal Behaviour and Physiology, School of Biological Sciences, Madurai Kamaraj University, Madurai 625 021, India

The circadian rhythm of locomotor activity of mouse pups is influenced by the duration of presence/absence of the mother in the 24-hour day. It was found that presence/absence (PA) cycles of 8:16 h, 10:14 h, 14:10 h and 16:8 h successfully entrained the activity rhythm of the pups whereas PA cycles of 6:18 h and 18:6 h failed to entrain. The results indicate that the duration of presence or absence of the mother must last at least 8 h of the standard 24 h periodicity to effectively entrain the rhythm of pups.

CIRCADIAN rhythms are daily rhythms which persist in the absence of time cues (*zeitgebers*) with a period length of approximately 24 h<sup>1</sup>. It is now well established that presence/absence (PA) cycles of mother mouse *Mus booduga* can act as a *zeitgeber* and entrain the circadian rhythm of locomotory activity of pups both in continuous darkness and in continuous light<sup>2-4</sup>. The pups took her presence to mean subjective day (rest hours) and her absence to mean subjective night (activity hours) perhaps mirroring the situation in nature. It is well known that light/dark (LD) cycles are the dominant and nearly universal *zeitgebers* of circadian rhythms<sup>5-7</sup>. The functional properties of entrainment of circadian rhythms in mammals by LD cycles have been extensively studied and well documented<sup>5,6,8</sup> whereas relatively little information is available on other *zeitgebers*. Daily pulses of short duration of just 10 seconds and of appropriate light intensities would be sufficient to entrain the circadian rhythm in mammals whereas pulses of darkness must last at least 2 h or more<sup>5,6,9-13</sup>. In this context it appears to be of interest to know what kind of cycles and what appropriate duration of presence and absence of the mother may bring about the entrainment in the activity rhythm of pups. Therefore in the present study a series of experiments were conducted to establish whether the 'duration ratio relations' of PA cycles have any resemblance to those of LD cycles.

## Methods

Pregnant animals of night active *M. booduga* were captured from the fields surrounding the Madurai Kamaraj University campus. They were kept in the light tight experimental cubicle (12' × 5' × 12'). The

cubicle was always maintained at constant temperature of 28 ± 1°C. The animals were divided into batches of 4-6 numbers. They generally littered 2-8 pups each. The day of birth was designated as day zero. Starting on day 5, two pups of either sex were selected from each litter, named A and B, and placed separately in plastic boxes of 21 × 15 × 13 cm. The mothers were cyclically presented alternately to the pups as described in Table 1.

Thus cycles of different presence and absence time ratios within 24 h were created. Since the pups (A and B) of the same litter were used in all series, a mother can create two different schedules of PA cycles. Thus if it is 6:18 for A it will be 18:6 for B and so on. In other words PA cycles to both pups were reciprocal. On day 16 the pups were introduced individually into activity running wheels and their locomotory activity was recorded using an A 620 X Esterline Angus Event recorder<sup>2-4</sup>.

During entrainment to PA cycles, the phase relationship between onset of a pup activity and the time of removal of mother was determined and averaged for each animal<sup>3</sup>.

The freerunning period ( $\tau$ ) was calculated by taking onset of activity as reference point, which, in the majority of records, exhibits high precision than the mid-point or the end of activity<sup>3</sup>.

Data presented in Table 2 were subjected to non-parametric statistical analysis using the Spearman rank correlation coefficient<sup>14</sup>.

Table 1. Experimental protocol.

Batch no.	Litter (n=4)	PA time ratio (h)	Time (h)
1	A	6:18	P=1200-1800 A=1800-1200
	B	18:6	P=1800-1200 A=1200-1800
2	A	8:16	P=1000-1800 A=1800-1000
	B	16:8	P=1800-1000 A=1000-1800
3	A	10:14	P=0800-1800 A=1800-0800
	B	14:10	P=1800-0800 A=0800-1800

**Table 2.** Mean period ( $\bar{\tau}$ ) after discontinuation of PA cycles with different PA time ratios.

Days	8:16 h		10:14 h		14:10 h		16:8 h		Spearman rank correlation coefficient ( $r_s$ ) of different PA time ratios and $\tau$
	<i>n</i>	$\bar{\tau} \pm SD$	<i>n</i>	$\bar{\tau} \pm SD$	<i>n</i>	$\bar{\tau} \pm SD$	<i>n</i>	$\bar{\tau} \pm SD$	
31-40	4	23.48 $\pm$ 0.30	4	23.18 $\pm$ 0.84	4	23.51 $\pm$ 0.48	4	23.63 $\pm$ 0.25	0.382*
41-50	3	23.37 $\pm$ 0.29	3	22.73 $\pm$ 0.53	3	23.40 $\pm$ 0.21	3	23.73 $\pm$ 0.20	0.517*
51-60	3	23.43 $\pm$ 0.28	3	22.64 $\pm$ 0.45	3	23.48 $\pm$ 0.22	3	23.68 $\pm$ 0.26	0.304*

\*Not significant at 5% probability level.

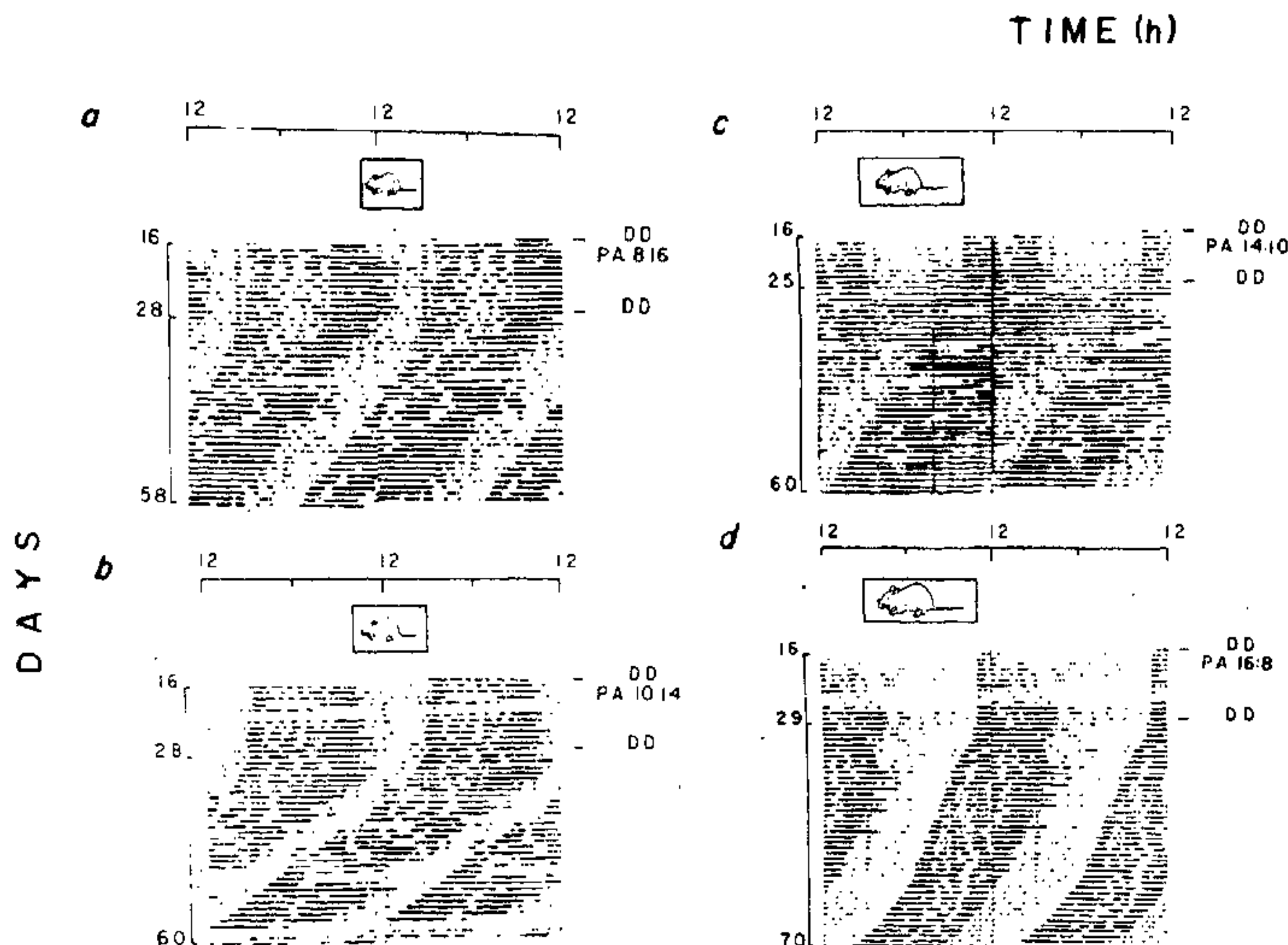
**Results**

Cycles of 8:16 h, 10:14 h, 14:10 h and 16:8 h successfully entrained the circadian activity rhythm of pups in DD (Figure 1a-d). Similarities in the type of entrainment have been noticed in all these cases. They are: (i) During the days of entrainment, the activity of most of the animals began immediately after the time of removal of the mother and was confined to the hours of mother's absence. The phase angle difference of the activity rhythm was positive and very small in the range of a few minutes (2-8 min). (ii) When the PA cycles were discontinued the rhythm started to freerun with the onset of activity coinciding again with the time of removal of the mother during the days of entrainment. The  $\tau$  of the freerunning rhythm of majority of the animals was <24 h. No correlation between  $\tau$  values and the different PA time ratios was found after discontinuation of PA cycles (Table 2;  $P > 0.05$ ).

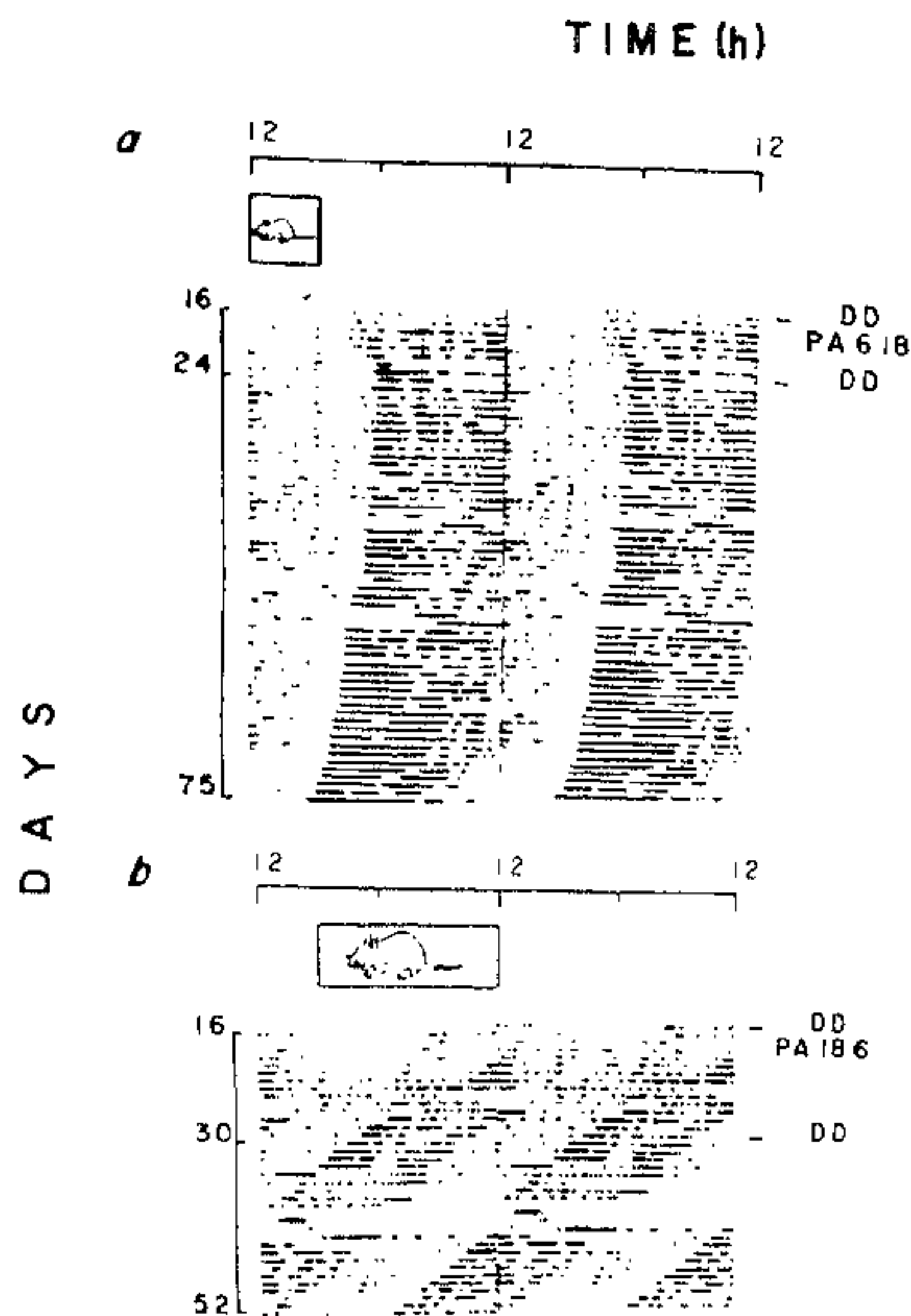
Figure 2 presents data of failure of PA cycles of 6:18 h and 18:6 h to entrain the activity rhythm of pups. The  $\tau$  of the rhythm is unable to equal the period of the PA cycles ( $T = 24$  h), if offered in the form of 6:18 h or 18:6 h schedules. However, the activity rhythm of pups freeruns. In both cases a gradual change in  $\tau$  was observed when day advances (Table 3).

**Discussion**

Entrainment of a circadian rhythm by a *zeitgeber* depends on phase control. Cycles of 8:16 h, 10:14 h, 14:10 h and 16:8 h of presence and absence of mother mouse entrain the circadian activity rhythm of pups (Figure 1a-d). The entrainment properties in these cases are similar to those brought out for entrainment by PA cycles of 12:12 h<sup>2,3</sup>. The present results indicate that the duration of presence or absence of the mother must last at least 8 h of the standard 24 h periodicity to



**Figure 1.** Double plotted wheel-running activity records of pups exposed to presence/absence cycles with different PA time ratios. **a.** 8:16 h; **b.** 10:14 h; **c.** 14:10 h and **d.** 16:8 h (the presence of mother is indicated by a bar). These cycles successfully entrain the activity rhythm.



**Figure 2.** Double plotted wheel-running activity records of pups exposed to PA cycles with different time ratios. **a**, 6:18 h and, **b**, 18:6 h (the presence of mother is indicated by a bar). These cycles failed to entrain the activity rhythm.

**Table 3.** Mean period ( $\bar{\tau}$ ) during and after PA cycles with different PA time ratios.

Days	6:18 h		18:6 h	
	<i>n</i>	$\bar{\tau} \pm SD$	<i>n</i>	$\bar{\tau} \pm SD$
16 20	4	24.29 $\pm$ 0.26	4	23.55 $\pm$ 0.07
21 30	4	23.95 $\pm$ 0.07	3	23.38 $\pm$ 0.87
31 40	4	23.97 $\pm$ 0.07	3	23.18 $\pm$ 0.40
41 50	3	23.90 $\pm$ 0.09	3	23.04 $\pm$ 0.19
51 60	3	23.91 $\pm$ 0.50	3	23.06 $\pm$ 0.26
61 70	2	23.87	2	23.04

effectively entrain the rhythm of pups. In the case of LD cycles a few hours, minutes or even seconds of appropriate light intensities would be enough to entrain the activity rhythm of several circadian systems. On the contrary darkness must last at least 2 h or more<sup>5,6,9-13</sup>.

In nature the mother mouse stays inside the burrow during light hours and forages during night. It is not known, how long the mother really interacts or is in physical contact with the pups within the natural 24 h cycle. In Madurai (9° 58' N, 78° 10' E) the day and night are equitable half-cycles, each half lasting roughly 12 h throughout the year. It may be inferred from the present results that at least 8 h of presence (PA 8:16) or 8 h of absence (PA 16:8) of the mother in a 24 h cycle might be needed for the altricial pups to maintain the phase relationship of the prenatally set clock/rhythm to

that of the environment. It was already reported in rats and squirrel monkeys that the mother acts as a transducer and co-ordinates the timing (phase) of the foetal biological clock to her own clock time, which in turn is entrained by ambient lighting<sup>15-17</sup>. While PA cycles of the mother mouse entrain the activity rhythm of the pups, the mechanism by which it does so remains to be determined.

Interestingly the PA cycles of 6:18 h (too little presence) and 18:6 h (too much presence) failed to entrain the activity rhythm of pups (Figure 2). However, their rhythms do persist but freerun as in constant condition. It is suggested that in these cycles the potency of the *zeitgeber* is reduced, which resulted in the freerunning pattern of activity rhythms. The changes of  $\tau$  with age as observed in these cases (Table 3) were similar to that of the earlier findings<sup>18-20</sup>. The period length of the activity rhythm of mice and hamsters shortens with age<sup>18,19</sup>. Davis and Menaker<sup>20</sup> reported the opposite effect— $\tau$  lengthening with age for *Mus musculus*. The subject of changes of  $\tau$  with age clearly calls for more research.

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