the first cell cleavage, probably sensitive to colchicine and also situated between pronucleus formation and the first cell cleavage. Further studies are necessary to define the precise role of prolyl endopeptidase in fertilization, and to search for the natural target materials (substrates) for the enzyme.

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Presence-absence cycles of the mother and not light-darkness are the zeitgeber for the circadian rhythm of newborn mice

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Summary. The relative roles of conflicting zeitgebers [presence/absence (PA) cycles versus light/dark (LD) cycles] on entrainment of circadian rhythm of locomotor activity were tested in pups of the night active mouse *Mus booduga*. During the early days of the pups' life the PA cycles of the mother acted as a zeitgeber and entrained their activity rhythm, even though the LD cycles were available. Entrainment by LD cycles took place only when the pups' eyes opened and probably became functional.
Key words. Maternal entrainment; circadian; zeitgeber; activity rhythm; *Mus booduga*.

In previous studies we demonstrated that presence/absence (PA) cycles of the mother mouse act as a zeitgeber and entrain the circadian rhythm of locomotor activity in *Mus booduga* pups under continuous darkness or continuous dim light [2–4]. Pups of this species, which is altricial and burrow-dwelling, rely behaviourally on the mother and take her presence as day and absence as night. Such mother/infant interactions probably help the pups to maintain the phase relationship of the prenatally set clock [5, 6] to that of the environment. In nature, the pups open their eyes on days 12–14 [2, 7], are weaned on around days 22–24 and start independent life at 30–35 days [8, 9]. It is well known that light/dark (LD) cycles are powerful and nearly universal zeitgebers of several circadian systems [10, 11]. In mammals the photic entrainment of the circadian clock occurs only via the retina during development [10–13]. Thus it appears that behavioural maternal entrainment could be expected to persist for a few days, until the time retina-mediated pathways become functional so that the developing clock can be synchronized directly to the physical zeitgebers such as environmental LD cycles. It is possible that during the early days of the pups' life a transition occurs in their circadian activity rhythm from a state of entrainment by PA cycles to a new state of entrainment again, this time by LD cycles. I have tested this hypothesis in the night active mouse *M. booduga*.

Materials and methods. Pregnant *M. booduga* were captured in the fields around the university campus. They were maintained in LD cycles (L: 06.00–18.00 h and D: 18.00–06.00 h) and produced litters of 2–8 pups each. The light intensity available to the animals was about 8–10 lx (Light source: incandescent bulbs – Philips, India). The animals for these experiments were a total of 8 mothers and 16 pups. The day of birth was designated as day 0. 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 presented for 12-h periods alternately to the pups, thereby creating PA cycles of 12:12 h. Thus the A pups were berthed with their mothers between 12.00–24.00 h and B pups between 24.00–12.00 h. Therefore the pups experienced behavioural and physical zeitgebers displaced relative to each other by 90° (figs 1 and 2).

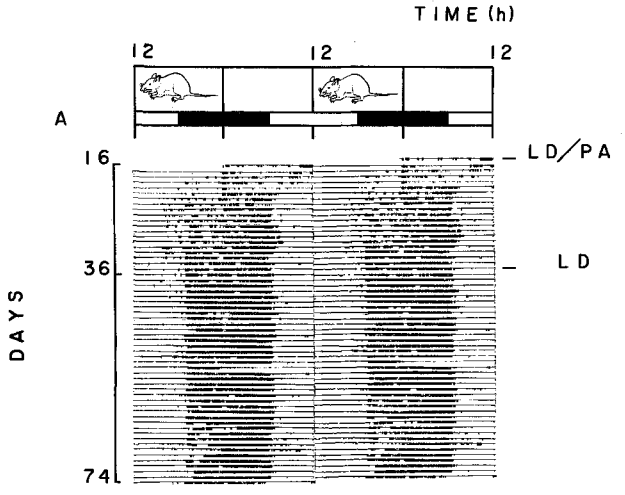

Figure 1. Double plotted wheel running activity of a pup exposed to conflicting zeitgebers (PA cycles versus LD cycles). Presence of mother: 12.00–24.00 h. L (open area) 06.00–18.00 h; D (shaded area) 18.00–06.00 h.

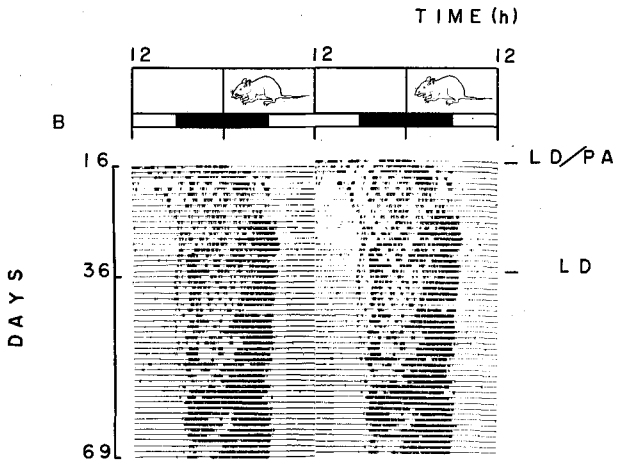

Figure 2. Double plotted wheel running activity of a pup exposed to conflicting zeitgebers. Presence of mother: 24.00–12.00 h. L (open area) 06.00–18.00 h; D (shaded area) 18.00–06.00 h. Note that the PA cycles in this experiment have been shifted 180° relative to that in the experiment illustrated in fig. 1.

Effect of PA cycles of mother and LD cycles on the circadian rhythm of pups during their first 16 days

	Pups A	B
Number tested	8	8
Number entrained to PA cycles	8	8
Percent (%) entrainment	100	100
Number entrained to LD cycles	0	0

Thus whereas the light came on at 06.00 h, the mother was presented only at 12.00 h for A and at 24.00 h for B. On day 16 the pups were introduced into running wheels and the locomotor activity was recorded using an A 620 X Esterline Angus event recorder[2–4]. The PA cycles were continued until day 36 but the mother was tethered by a 10-cm aluminium chain, barring her from entering the wheel. The mother/infant interactions thus took place in the nesting cage attached to the wheels. Food and water were available at all times.

Results and discussion. The effect of PA cycles of the mother and LD cycles on the circadian activity rhythm of pups is indicated in the table. The pups revealed onsets of activity on day 16 which were 180° off course relative to each other (figs 1 and 2). Thus the activity rhythm of A pups began at ~ 24.00 h (6-h phase delay with respect to the onset of darkness) and for B pups at ~ 12.00 h (6-h phase advance with respect to the onset of darkness). For each pup this onset of activity coincides with the time of removal of the mother. From these results it may be inferred that the activity rhythm of pups did entrain to the PA cycles for the first 16 days, even though the LD cycles were available. From day 17 onwards the influence of LD cycles on the activity rhythm becomes noticeable, i.e. the physical zeitgeber starts overriding the influence of the behavioural zeitgeber. As a consequence, the onset of activity as well as the offset of activity of each cycle drifted gradually towards the onset of darkness and the end of darkness respectively, until the entrainment by LD cycles ensued (figs 1 and 2). Such entrainment generally occurs approximately after days 20–22.

To the best of my knowledge this report is the first experimental demonstration of a circadian rhythm switching from a state of entrainment by a behavioural zeitgeber to a state of entrainment by a physical zeitgeber (figs 1 and 2). The results clearly indicate that the developing animals during their early days consider only the PA cycles of mother as zeitgeber even though the LD cycles were available. The PA cycles would probably provide the developing pups with a state of internal temporal order. The normal phasing and nocturnality in their activity pattern are determined by this behavioural zeitgeber. Such early behavioural maternal entrainment may have considerable physiological significance. Without PA cycles, the pups would develop circadian rhythms uncoordinated to the environmental LD cycles and render them vulnerable to environmental hazards like predation pressure etc.

The mouse requires functional eyes to entrain its activity/rest to LD cycles[10–13]. The absence of entrainment to LD cycles in the early days of pups' life could be due to the non-functioning of the eyes. When the eyes open, which usually occurs in this mouse around days 12–14[2,7], and probably become functional, they could perceive LD conditions and their activity rhythm would tend to be entrained to these (figs 1 and 2). In conclusion, during the early days of the pups' life the behavioural zeitgeber seems to play a dominant role compared with the physical zeitgeber.

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Effects of enterally- and parenterally-administered bombesin on intestinal luminal tryptic activity and protein in the suckling rat

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Summary. Because of the presence of bombesin-like immunoreactivity in milk, we investigated if enteral administration of bombesin affects the intestinal luminal content of trypsin and protein in 12–14-day-old rats. Bombesin (40 μg/kg), given either orogastrically or subcutaneously, produced a significant elevation in the intestinal content of trypsin activity. Thus, enterally-administered bombesin can produce acute biologic effects in suckling rats.
Key words. Bombesin; milk peptides; pancreas; trypsin; suckling rat.

Bombesin is a tetradecapeptide first isolated from the skin of the frog, *Bombina bombina*[1]. Subsequently, structurally and functionally homologous peptides have been isolated and characterized in a number of mammalian species[2]. Bombesin and bombesin-related peptides have been shown to be potent pancreatic secretagogues, both in vivo[3] and in vitro[4], and specific bombesin receptors have been demonstrated on the pancreatic acinar cell[5]. Substances with bombesin-like immunoreactivity and biologic activity[6–9] have recently been demonstrated in mild. Since other substances contained in milk have been shown to exert biologic activity in suckling animals when given orally[10], it seemed reasonable to hypothesize that the bombesin-like peptides found in milk might also be capable of eliciting biologic effects on the suckling animal.

We therefore conducted experiments seeking to determine if bombesin, administered orogastrically, exerted effects on pancreatic exocrine secretions of suckling rats. Specifically, changes in the intestinal luminal content of trypsin and protein were evaluated. A preliminary report of these findings has been presented[11].

Methods. Sprague Dawley rats raised in our own colony were used throughout these experiments. Animals were exposed to an alternating cycle of light and darkness for 12-h periods. The date of birth was set as day 0, and litter size was corrected to 10 on day 2 of life in order to standardize growth and development. The mothers and their pups had free access to water and standard chow (Lab-Blox, Allied Mills, Chicago, IL). The pups were allowed to suckle normally until day 12–14 when they were fasted 12 h and then studied. They were kept in cages with half of the floor placed on a heating pad to enable them to maintain their own normal body temperature[12]. Experiments were conducted within litters; rats in each litter were divided into three groups of equal body weight. The first group, designated 'control' received 0.1 ml of 0.9 N NaCl with 0.2% bovine serum albumin (Sigma, St. Louis, MO), designated 'SAL/BSA' by s.c. injection as well as orogastrically through a 3.5 French catheter. The second group was designated 'OG' since rats in this group received 0.1 ml of a solution containing 40 μg/kg of bombesin tetradecapeptide (Bachem, Torrance, CA) dissolved in SAL/BSA orogastrically through a 3.5 French catheter, and 0.1 ml of SAL/BSA by s.c. injection. The third group was designated 'SQ' since rats in this group received 0.1 ml of a solution containing 40 μg/kg of bombesin tetradecapeptide dissolved in SAL/BSA by s.c. injection and 0.1 ml of SAL/BSA orogastrically through a 3.5 French catheter.