

Mother mouse sets the circadian clock of pups

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Abstract. We report here the ontogeny of a circadian clock of the field mouse *Mus booduga* expressing itself 16 days after parturition in the locomotory activity of neonate pups removed from the mother and held in continuous darkness ever since birth. Locomotion is a 'complex' activity serving such functions as foraging, exploration, and territoriality. Since these functions are not conventionally associated with neonate and altricial animals, it is of interest that this ability has such an early circadian origin. A backward extrapolation of the pups rhythm and the rhythm of the mother strongly implicate maternal synchronization. The period of the circadian rhythm of the pups shortens with age, from birth up to six months.

Keywords. Ontogeny; circadian clock; *Mus booduga*; neonate pups; rhythm; maternal synchronization.

1. Introduction

Circadian clocks are now known to be innate, heritable and genetic in origin (Bünning 1977). Experiments with fruitflies (Pittendrigh 1954), lizards (Hoffmann 1957), chicken (Aschoff and Meyer-Lohmann 1954), mice (Aschoff 1960), rats (Browman 1952) and humans (Martin du pan 1974; Miles *et al* 1977) raised under continuous light (LL) or continuous darkness (DD) showed that animals need not experience light/darkness (LD) cycles during development in order to possess circadian rhythms.

Except for the first (fertilization: Everett 1961) and final (birth: Smolensky *et al* 1972) events of prenatal life the circadian clock appears to have no input to developmental timing in vertebrates. Even these circadian inputs should be credited to the circadian organizational account of the parent organisms. Primary circadian rhythmicity is predominantly a postnatal phenomenon (Davis 1981). Yet the study of the ontogeny of circadian rhythms in higher vertebrates is very important even if difficult. Much recent work with mice and rats confirms that rudiments of a circadian organization are already detectable in the fetal embryonic stage (Barr 1973; Fuchs and Moore 1980; Reppert and Schwartz 1983). The circadian organization specifically referred to serotonin acetylcoenzyme A N-acetyltransferase (Deguchi 1975), plasmacorticosterone (Levin *et al* 1976; Takahashi *et al* 1982) and suckling and feeding rhythms (Levin and Stern 1975). The functions assayed and documented above are very basic and intimately bound to life in the uterus and similar rhythms in the mother. In this paper we report the ontogeny of a circadian clock expressing itself in a complex function such as locomotory activity in the pups of the mouse *Mus booduga* and trace a maternal setting of this clock.

2. Materials and methods

Pregnant females of the field mouse *Mus booduga* were captured from fields surrounding the Madurai Kamaraj University Campus. The mother mouse was in

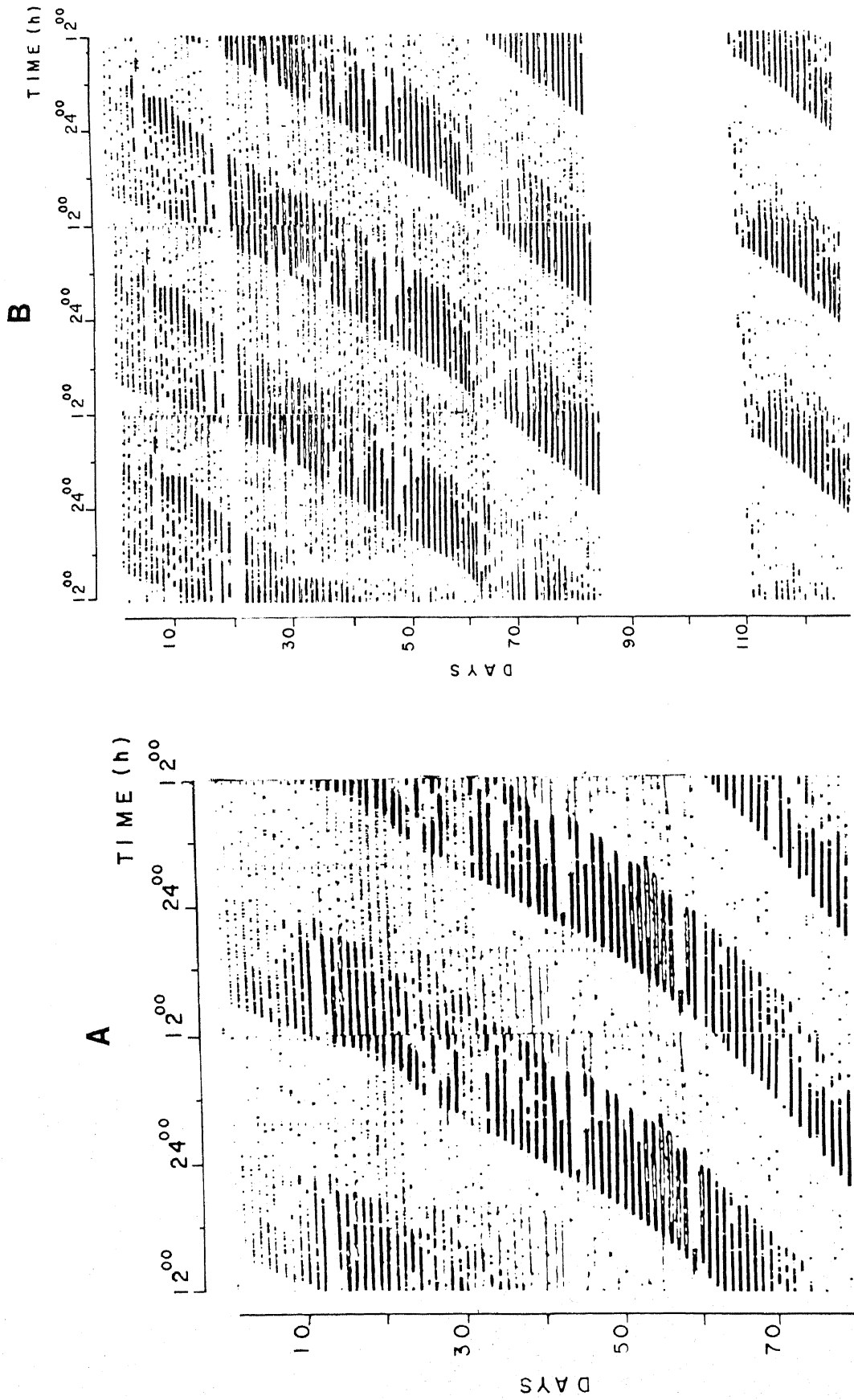


Figure 1. A & B

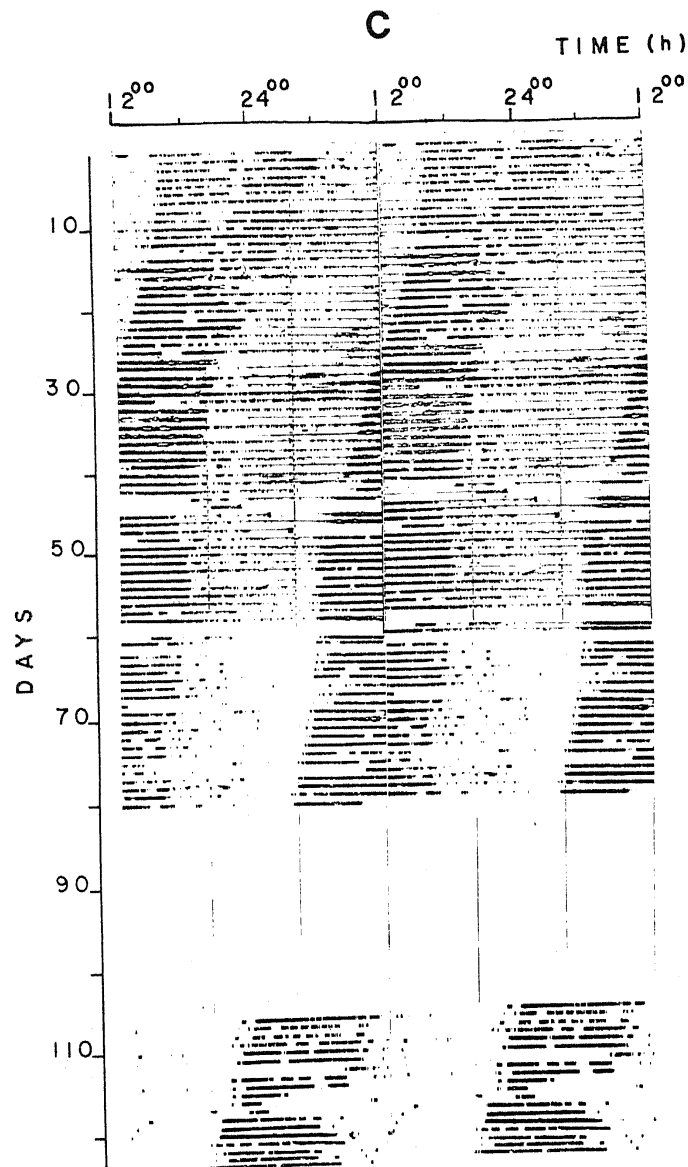


Figure 1. Recordings obtained with activity running wheel and an Esterline Angus event recorder for the locomotor activity in DD of **A.** mother and **B.C.** two pups of the field mouse *Mus booduga*. Actograms, constructed by pasting 24 hr strips of activity/rest data chronologically one below the other, are duplicated (triplicated in 1B) for easy visual estimation of onset/end of activity and other trends. Day 1 of the records is identical with day 16 of the animals after parturition. Activity monitoring was performed for 80 days (1A) in the mother mouse and for 128 and 124 days for the pups in 1B and 1C respectively. No activity monitoring was undertaken on days indicated by the gaps in the actograms for figures 1B, C.

continuous darkness (DD) for 4 days before littering and for another 80 days after the act of parturition. The pups are altricial with closed eyes and cling to the mother until they are 12–14 days old. They first open their eyes on 12th day after parturition. We could transfer the pups to delicately poised activity running wheels (used otherwise for recording the running activity of cockroaches) on day 16. The mother mouse and pups were fed with millets and grains and had access to water *ad libitum* in DD. Dim red light (> 610 nm) of $10 \mu\text{W cm}^{-2} \text{sec}^{-1}$ was used for cleaning the cages/wheels, transfer of

animals etc. Red light of this wavelength is 'safe' up to an intensity level of $150 \mu\text{W cm}^{-2} \text{sec}^{-1}$ in this animal (Viswanathan unpublished observations). The activity (running) rhythm of the mother was also measured using a sturdier running wheel. An eccentrically placed magnet of the wheel temporarily made and broke contacts in an electrical circuit with every turn of the wheel. The revolutions were picked up by channels of an A62OX Esterline Angus event recorder. Actograms were constructed and double/triple plotted in a manner which is now routine in chronobiological research.

3. Results

Figure 1A illustrates the locomotory activity pattern of the mother mouse starting 16 days after parturition for 80 days in constant darkness (DD). The rhythm is in a state of freerun and displays a period length of 23.47 hr. The onset of activity day after day is precise and the level and amount of activity are consistent and high. Figures 1B, C illustrate the locomotory activity patterns of 2 pups each 16 days old, born and maintained in DD. They were never exposed to any white light after their birth. Yet the rhythm in the locomotory activity is unmistakably present even on the first day of the experiment and persisted over the entire 4 month period of the activity recording. The rhythms freerun in the pups but the period length varies between the two; figure 1B showing a period of 23.42 hr and figure 1C a period of 23.76 hr. The onset of activity was used in computing the period length since it was much more stable than the midpoint of activity or the end of activity (Kenagy 1980; Chandrashekar *et al* 1983). By a process of backward extrapolation of the slopes of the locomotor activity rhythms of the two pups to the slope of the rhythm of the mother it becomes evident that all 3 clocks, that of the mother and those of the two pups, would have been in the same phase sometime about parturition. Since the pups had not been exposed to LD cycles at any time, and yet possess circadian rhythms, it must be presumed that they not only inherited them from the mother but also had it set (entrained) for them by her. Entrainment (prior to freerun) must have ensued *in utero* (Reppert and Schwartz 1983).

Figure 2 presents details of the changes undergone by the period length of circadian rhythm of pups as a function of age until they are 180 and 160 days old. The period length of the circadian rhythm of the pups decreases *i.e.* becomes shorter with age which is evident in both the cases.

4. Discussion

A circadian rhythmicity in the organization of activity/rest patterns in higher vertebrates and man seems to be a postnatal event. Human infants develop a sleep wakefulness rhythm with a circadian pattern only 3–4 months of their birth (Kleitman and Engelmann 1953). Furthermore it may be that circadian rhythms in most cases were not assayable in new-born animals because the functions being measured had not 'matured' (Davis 1981). The circadian systems might already be there before the circadian rhythm becomes overt. Deguchi (1975) pioneered an ingenious approach to assaying circadian organization in the foetus or in neonate rats. Deguchi monitored the circadian rhythm in serotonin N-acetyltransferase after it became overt and assayable

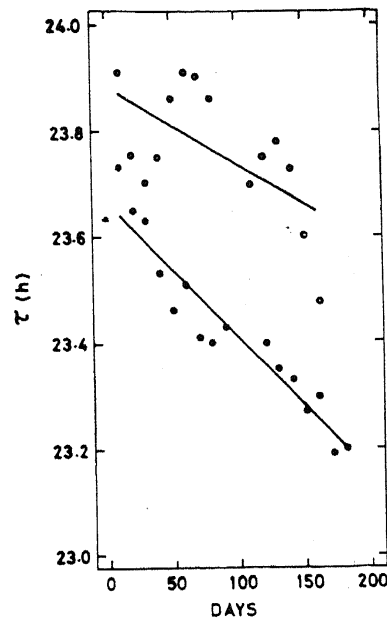


Figure 2. Changes of freerunning period (τ) of the locomotor activity rhythm with time in DD of two pups which had not seen any light since birth. The animals were 16 days old at the start of this experiment. The data points are for 10-day time slots for 180 and 160 days for pups 1B (●) and 1C (○) respectively. Linear regression lines are drawn showing significant decrease of τ with increase of age. $Y = 23.670 - 0.002X$; $r = 0.95$; $P < 0.001$ in one pup (●) and $Y = 23.888 - 0.001X$; $r = 0.62$; $P < 0.05$ in the other (○).

and used its phase to infer the state of the oscillator at the earlier developmental stages. Using the methodology of Deguchi (1975), Davis (1981) found no wheel-running rhythm in rats up to 11–15 days after which a circadian rhythm materialized. The onset of the activity of the pups occurred at the approximate time expected if they had been entrained to the mother. This is precisely what one obtains in the circadian rhythms of the pups of *Mus booduga* being described in this paper. For reasons of methodology we could not make altricial pups with eyes closed until the 12th day and entirely dependent on the mother, to run activity wheels. On day 16 when the pups could be separated, they were placed in activity running wheels in which they displayed a circadian clock timing of their activity. The backward tracing and extrapolation of phases of the pups and mother's activity rhythms strongly suggest maternal synchronization. For the rats studied by Deguchi (1975), Davis (1981) and our mouse, the entrainable principle precedes the overt circadian rhythms.

Reppert and Schwartz (1983) found that an entrainable circadian clock is present in the suprachiasmatic nuclei even during fetal development and that the maternal circadian system coordinates the phase of the clock. This finding lends further credence to our interpretation that the basic circadian oscillation underlying the locomotor activity rhythm of *Mus booduga* may have been present even in the fetus.

Further critical and detailed investigations alone can clarify how strong a zeitgeber the mother can be in entraining the rhythms of her progeny. It is interesting, however, that even foster mother rats seem to entrain the adrenocortical circadian rhythm in blinded pups. When blinded pups were raised by blinded mothers whose rhythm was out of phase with the LD-cycles, the rhythm of the pup was *in phase* with that of the mother's rhythm. Clearly the influence of the foster mother overrode the influence of

the lighting cycle (Takahashi *et al* 1982). In this report we are dealing with the appearance and expression of circadian rhythms in gross locomotor (wheel running) activity of the mouse *Mus booduga*. To this extent we are obviously dealing with the 'accomplishment of the organization of the circadian rhythm'. Locomotor activity is a highly integrated and complex activity serving functions as diverse as foraging, exploration, territoriality etc. These are functions that are not behaviourally associated with new-born altricial animals. It is in this context that the presence of this circadian ability at birth, is of interest. Another point is that most other fetal and prenatal rhythms reported have been measured *in utero* and hence may be rhythms simply imposed by the internal rhythmic environment of the mother (Barr 1973; Reppert and Schwartz 1983).

The period length of the infant *Mus booduga* clearly shortens with age (figure 2). The data points are for 10-day time slots for nearly 6 months. Pittendrigh and Daan (1974) have shown a similar shortening of the period of rhythm (τ) in mice and hamsters from puberty through maturity to old age. Davis and Menaker (1981) reported the opposite effect— τ lengthening with age for *Mus musculus*. It may not be prudent at this stage to link age with τ , interesting though the implications could be. The subject of the effect of age on the pacemaker clearly calls for more research.

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