well as a tool to investigate mechanisms underlying the antidepressant response.

Treatment of SAD with bright WL first used photoperiod extension (3-, 2-, or 1-h WL at dawn and dusk); these were all effective[5,6,11–16]. Later studies found that giving WL only in the morning, evening, or at midday also appeared to be effective[12–15,17,19–24]. However most studies have been carried out with a relatively small number of patients. A recent global analysis[18] of all studies of WL treatment of SAD patients until now (N = 185) has indicated that clinical remission obtained with photoperiod extension or morning WL is of the order of 50%, whereas all evening WL averaged a response rate of 35%. The latter corresponds to the average placebo response rate found in atypical depression[18].

Thus although the pathogenesis of SAD and the mechanism of action of WL in improving the symptoms of SAD is not known, the findings that photoperiod extension is not necessary for light to exert beneficial effects indicate that the seasonal model invoked in its inception may no longer be valid. Morning light appears more efficacious than evening light[18,24]; further studies with low duration of WL exposure are needed to establish whether early morning is indeed the most sensitive circadian phase.

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Continuous light abolishes the maternal entrainment of the circadian activity rhythm of the pups in the field mouse

N. Viswanathan and M.K. Chandrashekaran

Department of Animal Behavior, School of Biological Sciences, Madurai Kamaraj University, Madurai 625021 (India), 13 August 1986

Summary. 12:12-h cycles of presence and absence of mother mouse act as a 'zeitgeber' and entrain the circadian rhythm of locomotor activity in the pups of *Mus booduga* under continuous darkness or continuous dim light. Continuous higher illumination of 15–25 lx abolishes this impressive maternal entrainment.

Keywords. Maternal entrainment; freerun; period; circadian pacemakers; *Mus booduga.*

Most studies on circadian behaviors in mammals have been restricted to adult animals in which the pathway of entrainment by environmental light and darkness (LD) is exclusively through the eyes[2,3]. In the infants of mice and rats the mother further acts as a transducer and coordinates the timing (phase) of the developing biological clock to her own clock time which, in turn, is entrained by ambient lighting[4–6]. We reported previously for *Mus booduga* that cycles of presence and absence of the mother mouse (PA cycles) entrain the circadian locomotor activity rhythm of pups both in continuous darkness (DD) and in continuous dim light (LL)[7]. However, some of our experiments (unpublished data) revealed that entrainment to PA cycles in LL of 3-10 lx was somewhat wobbly. LL is known to bring about radical alterations in circadian features[8] and even induce arrhythmia, split rhythms, etc.[9–11]. It has also been reported from this laboratory that social cues which synchronized the circadian flight activity rhythm of members of a colony of *Hipposideros speoris* bats in DD failed to do so in LL of 10–20 lx[12]. We therefore performed experiments to investigate whether LL of appropriately high intensities would in any manner impair the maternal entrainment of the circadian activity rhythm of pups.

Materials and methods. Pregnant mice *M. booduga* (17 in number) were captured from the fields around the University campus. Seven of them were maintained in DD and the other 10

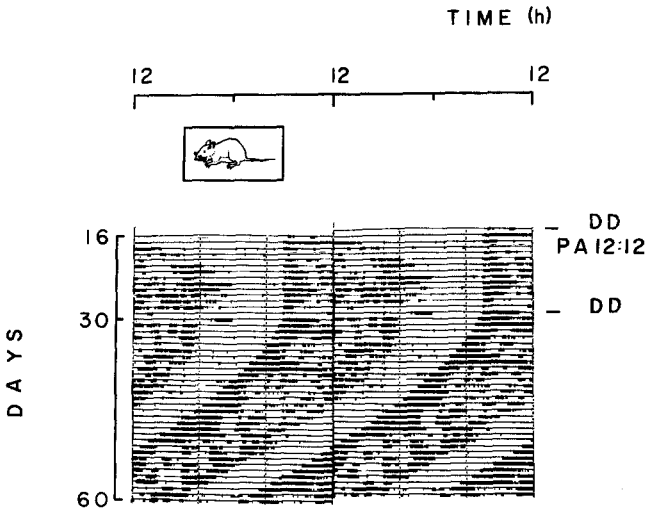

Figure 1. Continuous record of wheel running activity of a pup: days 16–30, DD and PA cycles of mother 12:12-h; days 31–60, DD. The record has been duplicated for easy visual estimation. Presence of the mother: 18.00–06.00 h. The presence of the mother is indicated by a bar.

animals in LL of 15–25 lx (incandescent light). They littered 2–8 pups each. Two pups of either sex were selected from a litter and placed in plastic boxes of 21 × 15 × 13 cm. Starting on day 5 of the pups' life, mothers were alternately presented and removed for 12 h thereby creating PA cycles of 12:12 h. Thus each of the two pups would be with the mother for one half of the cycle. On day 16 the pups were introduced into activity running wheels and the locomotor activity was recorded using an A620 X Esterline Angus Event Recorder. The PA cycles were continued but the mother was tethered by a 10-cm aluminium chain barring her from entering the running wheel. The mother/infant interactions thus took place in the nesting cage attached to the wheels. Food and water were provided ad libitum.

Results and discussion. Figure 1 illustrates an ideal case of maternal entrainment of the circadian rhythm in the locomotor activity of a pup in DD. In this behavioral entrainment the presence of the mother is apparently construed by the pup as subjective day and her absence as subjective night. The pup ran the wheel during subjective night. This entrainment of the pups by PA cycles has been reported by us earlier[7]. Figure 2 shows that this impressive maternal entrainment is abolished by higher LL and the circadian activity rhythm of pups freeruns with a τ of > 24 h in spite of PA cycles. The loss of such entrainment is obviously due to the action of light. Light is known to have dramatic effects on the endocrine system[13]. While the physiological basis underlying the ability of LL to abolish entrainment by PA cycles is not known, light probably uncouples the circadian pacemakers by altering the hormonal profiles[14,15]. This is, of course, at best a conjecture. Earlier findings of other authors that the effectiveness of maternal entrainment of infant rhythms can be markedly manipulated by LD cycles[16,17] and our present report that maternal entrainment can even be totally abolished by higher intensites of LL are of obvious interest for human maternity ward situations.

A further feature of interest in figure 2 is that the onset of activity of the pups on day 16 coincides with the time at which the mother was removed in the preceding days. This finding suggests that the pups did entrain to the PA cycles for the first 14–16 days notwithstanding higher LL intensities. Our explanation follows the law of parsimony: higher LL does not impair the maternal entrainment of locomotor activity rhythm of pups because light perception in these mammals is solely through the eyes[2,3] which open only around 12–14 days[18].

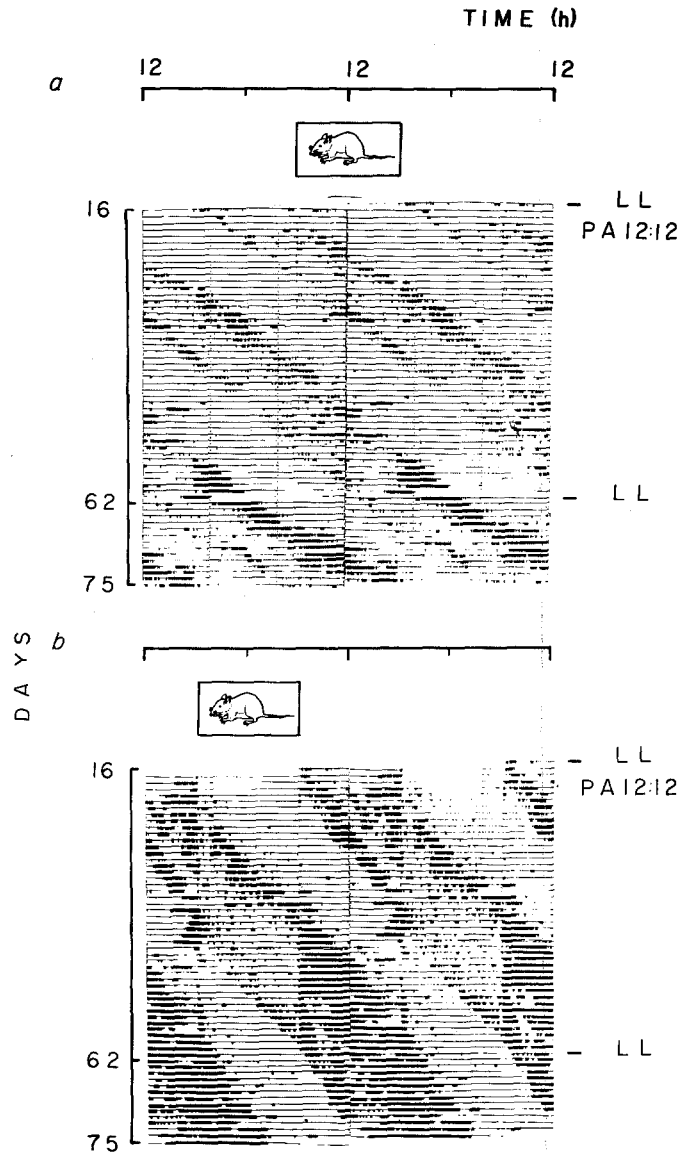

Figure 2. Continuous record of wheel running activity of the pups: **a** and **b** Days 16-62, LL and PA cycles of mother 12:12 h: days 63–75, LL. The record has been duplicated for easy visual estimation. Presence of mother τ with pup **a**) 06.00–18.00 h; pup **b**) 18.00–06.00 h. The presence of mother is indicated by a bar. The activity rhythm freeruns with a period (τ) of > 24 h (**a** τ = 24.61; **b** τ = 24.49). The periods of activity rhythms are not significantly different both during and after PA cycles.

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Debriding ability of a novel multi-enzyme preparation isolated from Antarctic krill *(Euphausia superba)*

D. Campbell, L. Hellgren*, B. Karlstam and J. Vincent*

*Pharmacia AB, S-751 82 Uppsala (Sweden), and *Department of Dermatology, University of Umeå, S-901 85 Umeå (Sweden),*
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Summary. The wound-debriding activity of various types of proteolytic enzymes and proteases from Antarctic krill (multi-enzyme system consisting of both endo- and exopeptidases) was evaluated. The results, based on the enzymatically achieved weight reduction of a necrotic animal material (excised rat skin) in vitro, clearly showed that the multi-enzyme system (krill) had a higher degrading activity than the single enzyme preparation, or that with only a few enzymes. The debriding effect of the krill enzymes was markedly related to the enzyme concentration, resulting in 70–100% substrate degradation after 24 h. The digesting capacity of trypsin reached about 50%, but an increase in concentration of this enzyme did not substantially influence its overall activity. The effect of streptokinase-streptodornase, collagenase and plasmin-desoxyribonuclease was weak (10–20% digested).
Key words. Enzymatic debridement; proteolytic enzymes; Antarctic krill *(Euphausia superba)*; trypsin; streptokinase-streptodornase; collagenase; plasmin-desoxyribonuclease.

The objective of enzymatic debridement in the secondary healing leg ulcers is to decompose the slough and/or necrotic tissue in the wound in order to achieve a 'red' granulating and epithelializing wound surface.

Proteolytic enzymes used for this purpose are heterogeneous as regards their origin, structure and substrate specificity. For evaluation of their in vitro activity a careful choice of clinically relevant substrates is of great importance. Therefore, in previous studies, we used substrates originating from human secondary ulcers (necroses, fibrin, blood clots)[1–3].

In the present report a necrotic animal model (excised rat skin) was chosen as a representative and reliable material for testing the potency of various enzymes. This tissue is very similar to, although not identical with the human necrosis which exists in different stages of dehydration/degradation.

The effects of the main enzymatic debriders used in Scandinavia, crystalline trypsin (Trypure®, Novo), streptokinase-streptodornase (Varidase®, Cyanamid), collagenase (Iruxol®, Knoll) and plasmin-desoxyribonuclease (Fibrolan®, Parke-Davis), were compared with that of a novel multi-enzyme preparation originating from Antarctic krill *(Euphasia superba)*.

One of the most characteristic features of this reddish shrimp-like crustacean is its rapid autolytic degradation post mortem. This self-deterioration is mediated by the activity of endogenous enzymes, especially the peptide hydrolases. These enzymes have recently been isolated and characterized, and their relevance in protein degradation reviewed[4]. The enzymes hitherto identified include three trypsin-like serine proteases of which one seems to possess a significant exopeptidase effect in addition to endopeptidase activity. The other two trypsin-like enzymes are true endopeptidases. Moreover, five enzymes exhibiting exopeptidase activity were identified and purified: two carboxypeptidases of the A-type, two carboxypeptidases of the B-type, and an aminopeptidase.

Material and methods. Enzyme preparations: The krill *(Euphausia superba)* raw material, originating from Japanese commercial catches (Taiyo Fishery Co.), was frozen aboard and kept at −20 °C until used. The krill proteases were isolated from a defatted aqueous extract, gel chromatographed and freeze-dried.

Commercially available crystalline trypsin (Trypure®, Novo), streptokinase-streptodornase (Varidase®, Cyanamid), collagenase (Iruxol®, Knoll) and plasmin-desoxyribonuclease (Fibrolan®, Parke-Davis),were used for comparison.

Determination of proteolytic activity: Comparisons of the proteolytic activity between Trypure® and the krill enzyme preparation were established by using two different biochemical enzyme assays.

The total proteolytic activity was determined with denatured casein as a substrate[5]. The enzymatic reaction – performed in 0.1 M Tris-HCl buffer, pH 7.5 – was terminated after 20 min by the addition of 5% trichloroacetic acid for precipitation of protein. After high-speed centrifugation and removal of the sediment, the supernatant was measured spectrophotometrically at 578 nm for the analysis of free aromatic amino acids using the Folin-Ciocalteau reagent. A tyrosine standard was used as reference

Enzymatic degradation of animal necrotic tissue (excised rat skin) in vitro at 37 °C and for 24 h (wet weight)

	20 mg/ml	10 mg/ml	5 mg/ml
Krill			
Weight before	66 mg	60 mg	69 mg
Weight after	0	0	18 mg
	−66 mg	−60 mg	−51 mg
Digested	100%	100%	74%
Trypure®			
Weight before	69 mg	63 mg	58 mg
Weight after	29 mg	29 mg	29 mg
	−40 mg	−34 mg	−29 mg
Digested	58%	54%	50%
Varidase®			
Weight before	69 mg	58 mg	59 mg
Weight after	61 mg	47 mg	46 mg
	− 8 mg	−11 mg	−13 mg
Digested	12%	19%	22%