cells in a motile state. Amiloride, which blocks Na^+ influx into cells, may lead to arrest of this process. The dose response curve relating amiloride concentration to sperm motility revealed an IC_{50} of about 5×10^{-5} M. This value is high when compared to those relating the inhibition of amiloride to Na^+ transport in other mammalian epithelia[8,10-12].

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Functional absence of brain photoreceptors mediating entrainment of circadian rhythms in the adult rat

G.A. Groos and D. van der Kooy

Department of Physiology and Physiological Physics, University of Leiden, Wassenaarseweg 62, 2300 RC Leiden (Netherlands), 8 April 1980

Summary. The photic energy penetrating into the brain was increased in adult rats sustaining craniotomies sealed with transparent plastic. After blinding, these animals failed to entrain their circadian food intake rhythm to light-dark cycles. Short pulses of light did not phase-shift the freerunning rhythm. We conclude that adult rats lack brain photoreceptors mediating entrainment of circadian rhythms.

In submammalian vertebrates photic entrainment of circadian rhythms can be mediated by an extraretinal photoreceptive system in the brain[1]. In mammals, on the other hand, blinding results in freerunning rhythms suggesting that entrainment is exclusively mediated by the retina[2]. However, substantial amounts of light penetrate into the brains of mammals[3]. Moreover, light impinging directly on hypothalamic cells can evoke photo-neuro-endocrine reflexes in blind adult rats[4]. It has also been shown that the pineal serotonine-N-acetyltransferase activity rhythm of blinded neonatal rats is entrained to the light-dark cycle. The photoreceptors for this entrainment lie within the animal's brain[5]. Since pineal photoreceptors have not been demonstrated in the rat[6], and, accordingly, pinealectomy in this species does not interfere with entrainment[7] these findings suggest a functional role for hypothalamic photoreceptors mediating entrainment in immature rats. This led us to investigate whether the circadian food intake rhythm of blinded adult rats can be entrained if the amount of light entering the brain is increased.

Materials and methods. In 20 adult male rats blinded by binocular enucleation the circadian rhythm of food intake was continuously recorded by monitoring the number of food approaches in 30-min intervals[8]. Throughout the experiment the animals were exposed to light-dark cycles (L:D 12:12 h; L = 300–400 lx). In 5 additional blind animals a hole was drilled in the skull at or just rostral to the bregma, exposing over 28 mm^2 of brain surface. The holes were sealed with a thin layer of transparent plastic. Thus light could penetrate directly into the brain. At a later stage of the experiment the animals sustaining such craniotomies were exposed to intense 1-h (white) light pulses (1600 $\mu W \cdot cm^{-2}$) at various phases of their circadian cycle. For this purpose they were anaesthetized with Hypnorm (Philips-Duphar, 1 $ml \cdot kg^{-1}$) and placed with the cranial opening in the beam of light. Control craniotomized animals were merely anaesthetized at corresponding phases.

Results and discussion. The 20 animals without craniotomies exhibited freerunning circadian food intake rhythms after binocular enucleation with periods exceeding 24 h (mean: 24.27 h; range: 24.09–24.47 h). Exposure to the L:D 12:12 lighting cycle therefore did not result in entrainment. Moreover, for none of the animals was evidence for passing synchronization obtained[9]. These findings are illustrated for a blinded rat in figure 1, B. Similarly, the rhythms of the craniotomized rats were freerunning with comparable periods (mean: 24.21 h; range: 24.07–24.38 h, e.g., figure 1, A). To further investigate the possible presence of an extraretinal photoreceptive system the brains of the craniotomized animals were illuminated with single 1-h light pulses of high intensity (1600 $\mu W \cdot cm^{-2}$). In sighted rats kept under conditions of constant illumination such pulses are known to result in phase shifts of the freerunning rhythm, the direction and magnitude of which systematically depend on the phase of the circadian cycle at which the light pulse is delivered[10]. Figure 2 summarizes the results of

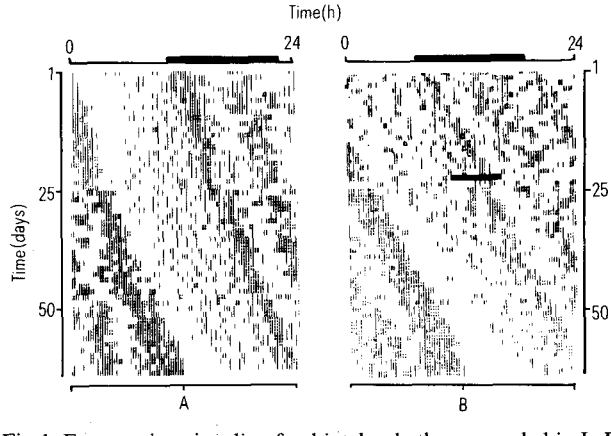

Fig. 1. Freerunning circadian food intake rhythms recorded in L:D 12:12 (as indicated above the records) for *A* a blinded craniotomized and *B* a normal blinded rat. The dark rectangle on day 23 in record B indicates the time and duration of control Hypnorm anaesthesia.

this experiment. Phase shifts were computed according to the linear regression method[11]. Single light pulses did not induce phase advances or delays. This finding holds irrespective of whether the beginning or the end of the circadian increase of food intake is taken as a phase reference. In order to test for metabolic and pharmacological effects of Hypnorm, craniotomized rats were anaesthetized at various phases of their cycle but not exposed to light pulses. It was observed that Hypnorm did not alter the period or phase of the freerunning rhythm (figures 1,B, and 2). It is conceivable that the anaesthesia reduced the sensitivity of the putative hypothalamic photoreceptors in these light pulse experiments. This interpretation is not supported, however, by electrical recordings from directly illuminated unanaesthetized slices of hypothalamic tissue showing the absence of photoreceptors[12]. Moreover, in view of the high intensity of the pulses it is significant that no response was observed at all.

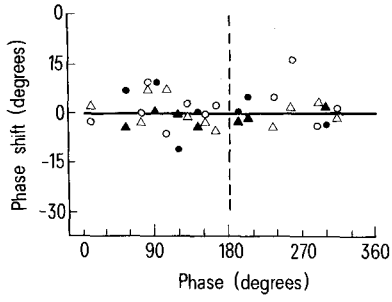

Fig. 2. Absence of light pulse induced phase shifts of the freerunning food intake rhythm of craniotomized rats. The period of each rhythm before the 1-h pulse was normalized to 360 degrees. Light pulses on just Hypnorm anaesthesia were delivered at different phases of the cycle. The phase shifts computed from linear regression analysis of eating onset times (circles) and times at which eating was terminated (triangles) are expressed in degress for light stimulated (open symbols) and control craniotomized rats (filled symbols). Phase 180° corresponds to the onset time of the circadian eating bout.

The present experiments show that increasing the photic energy penetrating into the exposed brain of blinded adult rats is ineffective in entraining the circadian food intake rhythm to daily light-dark cycles. Similar results were obtained in blinded animals without craniotomies. Our findings are in contrast with descriptions of extraretinal photoentrainment in neonatal rats[2,3] and the induction of photo-neuroendocrine reflexes by direct illumination of hypothalamic cells of the adult rat[4]. However, electrophysiological experiments showed the absence of such hypothalamic photoreceptors in adult rats[12]. Our observations suggest that extraretinal photoreceptors mediating entrainment of circadian rhythms in the rat, although present in immature animals, lose their functional significance at a later stage of life. This process may be correlated with the postnatal development of the retino-hypothalamic projection which is known to mediate entrainment in adult rats[2,13].

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Circadian variation of diazepam acute toxicity in mice

F.H. Ross, A.L. Sermons, J.O. Owasoyo[1] and C.A. Walker[2]

School of Pharmacy, Florida A & M University, Tallahassee (Florida 32307, USA), 4 March 1980

Summary. The LD_{50} of i.p. injected diazepam was determined every 4 h over a 24-h period in albino mice adapted to a 12-h dark/12-h light programmed illumination cycle. Results show that diazepam is more toxic during the light phase of the cycle than during the dark phase and demonstrate circadian variation in the toxicity of the compound in mice.

Several investigators have reported circadian variation in the toxicity and effectiveness of CNS drugs. It has been demonstrated that the so-called 'sleeping time' in mice after hexobarbital shows circadian variation[3]. Previous studies have shown that cholinergic drugs[4] as well as amphetamine[5] are more toxic during the dark phase of a light-dark cycle in rodents. It has also been found[6] that some adrenergic stimulants and blockers show opposite circadian patterns of toxicity in mice, in that while the adrenergic stimulants are more toxic during the dark phase of a light-dark illumination cycle, adrenergic blockers are, expectedly, more toxic during the light phase of the cycle. The circadian toxicity patterns of pentylenetetrazol, picrotoxin and sodium phenobarbital, and the ability of exogenous L-dopa, serotonin and gamma-amino-butyric acid (GABA) to alter those toxicity patterns have been reported[7]. While circadian variation in drug effectiveness and toxicity in mammals has not been fully explained, some investigators have suggested that these diurnal variations in CNS drug response and toxicity might be related to the endogenous brain levels of biogenic amines which have also been shown to vary diurnally[5,8-11]. The purpose of the present study was to investigate the circadian toxicity pattern of diazepam, a tranquilizer, in mice adapted to a programmed light-dark illumination cycle.

Methods. Male, Swiss-Webster mice weighing approximately 25 g each were used in this study. All animals were adapted, at a temperature of 23 ± 1 °C, to an environmental