

Short communication

Functional absence of extraocular photoreception in hamster circadian rhythm entrainment

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Abstract

The mammalian circadian pacemaker is entrainable by light via the retina. The putative role of extraocular light perception was investigated in blinded hamsters. These animals were shaved and exposed to a light-emitting pad for either 30 min or 3 h. The absence of any phase-shifting effects on wheel running activity rhythms indicates that extraocular light perception plays no functional role in photic entrainment of the circadian pacemaker in the hamster. © 1999 Elsevier Science B.V. All rights reserved.

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Daily variations in many physiological and behavioural functions are regulated by central circadian pacemakers. The anatomical localization of these pacemakers varies among invertebrate and vertebrate species [11]. In mammals, the primary biological clock resides in the suprachiasmatic nuclei (SCN) at the base of the hypothalamus [8,10].

The most important external stimulus to entrain the circadian pacemaker to the environmental cycle is light [5]. There is ample evidence that extraretinal photoreceptors are not functionally present in the mammalian brain [9,13]. Instead, light information reaches the SCN via the retina and the retinohypothalamic tract [6]. Recently, it has become a matter of debate whether illumination of the skin induces secondary processes that are able to affect the mammalian circadian system [1,2,4]. Campbell and Murphy [1] reported that in humans, skin illumination of the area behind the knee joint affects the circadian temperature and melatonin rhythm, with the effect being dependent on the phase of the circadian cycle. Light exposure during the beginning of the night resulted in phase delays of the rhythm, whereas at the end of the night, it resulted in phase advances. Retinal illumination in both nocturnal and

diurnal species, including humans, induces similar time-dependent phase shifts.

In search of the photoreceptors that mediate photoentrainment, various photopigments have been considered as candidates for the molecular basis of photoreceptor systems. The finding that cryptochromes are present in mammalian retinal ganglion cells, in the SCN, and in many other tissues including the human skin has extended the discussion on extraocular photoreception [7,12].

We examined the effect of skin illumination on the circadian system of the hamster, the general model for circadian rhythm research in mammals. Its very precise onset of running wheel activity (daily variation less than several minutes) provides a well-defined phase marker and therefore, the occurrence of phase shifts is clearly evident. Moreover, the use of this animal allows recordings over prolonged periods so that the presence of permanent phase shifts can be assessed with great reliability.

Twenty-six sighted hamsters were first exposed to a light pulse according to a standard protocol (Fig. 1a,b). In these nocturnal animals, the activity onset at around the beginning of the night is conventionally defined as circadian time (CT) 12. Light pulses were aimed either at CT 14 (2 h after activity onset) or at CT 19 (7 h after activity onset), the phases of the circadian cycle where phase delays and advances are maximal. The magnitude of the phase shifts were estimated by fitting straight lines through the onsets of activity before and after the light pulse, once

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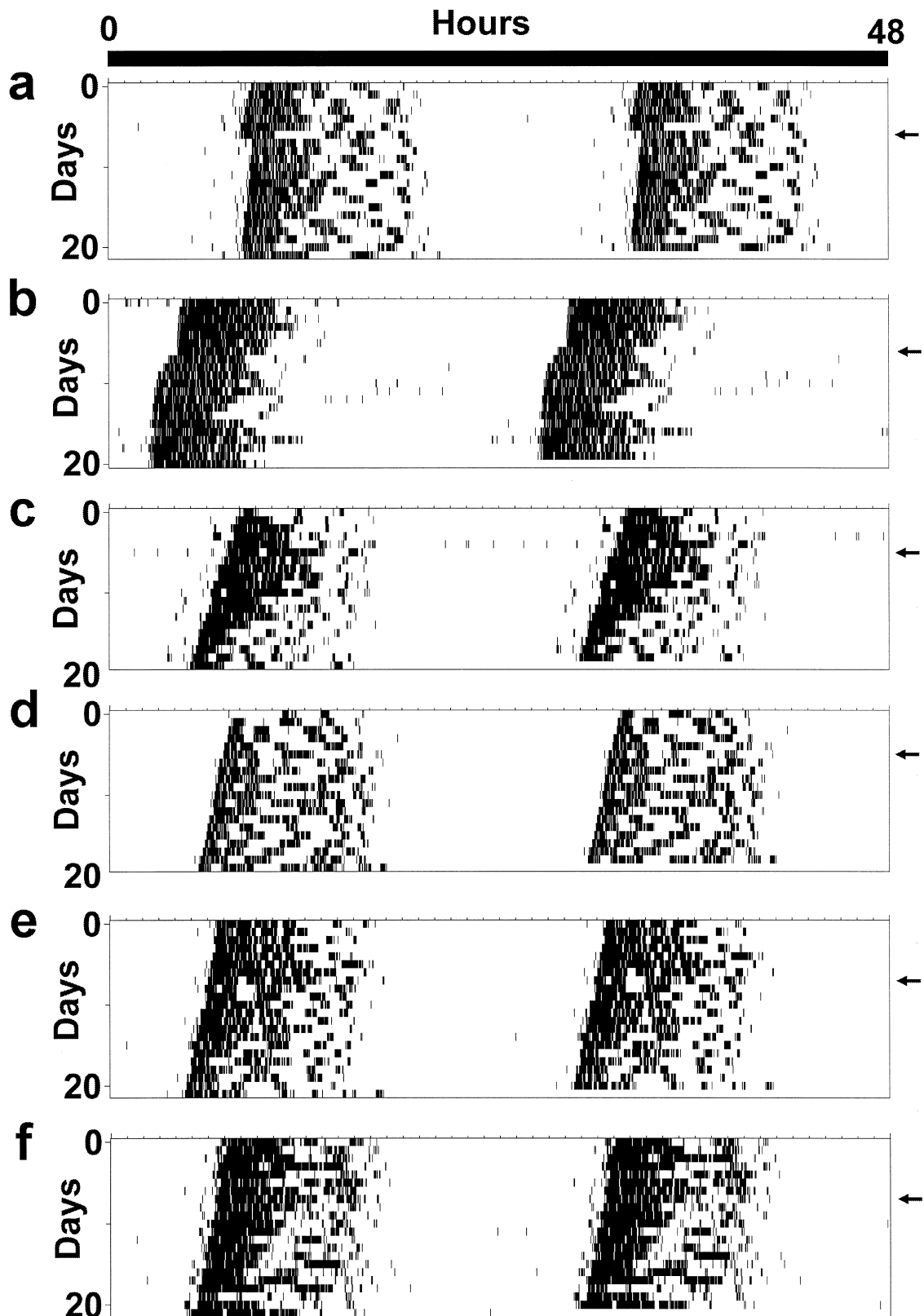


Fig. 1. Effect of light on circadian running wheel activity in hamsters. (a,b) The running wheel activity rhythms of the hamsters were entrained to a light dark cycle (L:D = 14:10) and were recorded with a resolution of 1 min. After release in constant darkness for 7 days, the animals received a 15-min light pulse (100 lux, day indicated by an arrow) either at CT 14 (a) or CT 19 (b). (c,d) Blinded hamsters were put on a BilliBlanket pad (about 10 cm × 15 cm) for 30 min at CT 14 or CT 19. (e,f) Blinded hamsters were put on the BilliBlanket pad for 30 min at CT 14 and CT 19, but the light source was not switched on.

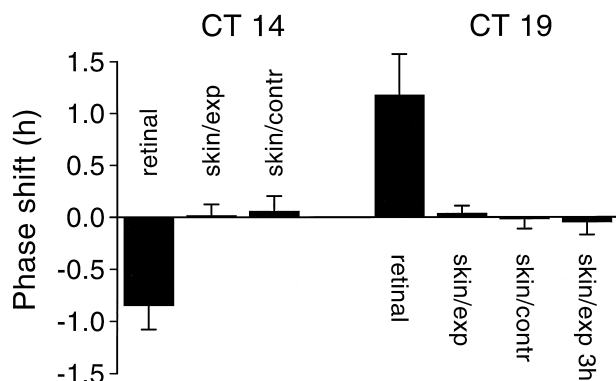


Fig. 2. Phase-shifting effects of light. Mean phase delays and advances of sighted animals at CT 14 ($n=14$) and CT 19 ($n=12$) following a 15-min light pulse (retinal). Mean responses of blinded animals following 30 min light exposure (skin/exp) or control treatment (skin/contr) on the BilliBlanket pad at CT 14 ($n=10$) and CT 19 ($n=10$). Mean phase shift following a 3-h light exposure to the BilliBlanket pad (skin/exp 3h) at CT 19 ($n=6$).

a stable freerunning rhythm was regained. The lines were extrapolated to the day after the light pulse to measure the magnitude of the phase delays (–) and advances (+). Each of the animals responded to the pulse at CT 14 with a delay (range: -0.5 – -1.3 h, $n=14$) and at CT 19 with an advance (range: 0.7 – 2.1 h, $n=12$). It was concluded that these hamsters displayed normal phase shifting responses to light pulses.

The hamsters were blinded according to a standard procedure [3] to investigate extraocular photoreception. The animals were kept in constant darkness for at least 7 days before they were put on a light-emitting pad for 30 min (Fig. 1c,d). The pad covered the entire bottom of a small cage and the animals could walk freely during the light pulse. The light source (BilliBlanket Plus, Ohmeda) was the same as that used by Campbell and Murphy [1]. The lower part of the body had been shaved to optimize illumination of the skin. This experiment was performed either at CT 14 ($n=10$) or at CT 19 ($n=10$). The procedure was repeated after 14 days with the light source switched off (Fig. 1e,f).

Extraocular light application at the beginning and end of the night resulted in phase changes of 0.02 ± 0.10 h (mean \pm S.D., $n=10$) and 0.04 ± 0.07 h ($n=10$), respectively (Fig. 2). The control experiments at these times resulted in mean phase changes of 0.06 ± 0.14 h ($n=10$) and -0.02 ± 0.09 h ($n=10$). None of the changes differed significantly from zero. A paired *t*-test between the experimental and control procedures revealed no significant differences. Six additional animals were exposed to a 3-h light pulse at CT 19. The mean shift induced was -0.05 ± 0.12 ($n=6$) h, which is not significantly different from zero.

The lack of any effect of skin illumination on the circadian system is evident despite the use of the same light source as in the experiments of Campbell and Mur-

phy [1]. Moreover, the BilliBlanket light source emits light in a range (400–550 nm) that covers the maximum absorption of the cryptochromes (420–430 nm) and can activate photoreceptors that are probably present in the hamster skin. It cannot be excluded, however, that the threshold for skin entrainment is lower when the eye pacemakers are present, but we consider this very unlikely. Our data clearly show that direct illumination of the skin does not result in phase shifts in hamsters. Therefore, the role of non-ocular phototransduction in photic entrainment does not extend to all mammals.

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