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A human phase–response curve to light

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Using 'classical' experimental protocols, a human phase–response curve (PRC) to a single 3-h bright light pulse has been established. When the light pulse was centred slightly before the time of body temperature minimum, the circadian system delayed, whilst a pulse slightly after the minimum advanced it. Maximum phase shifts were about 2 h. When light pulses over 3 successive cycles were used, larger shifts (4–7 h) were produced. It is concluded that the human PRC does not differ in principle from that found in other species, except with respect to the light intensity required.

Circadian rhythms are endogenous to all eukaryotic organisms. They are synchronized to the solar day by rhythmic external factors, or 'zeitgebers', the most important of which is the external light–dark cycle. The mechanism by which the circadian clock is synchronised by light is essentially non-parametric [21]. That is, a single light pulse given to an organism under constant conditions without time cues immediately resets the clock by an amount dependent upon the time of day at which it is given. The relationship between the timing of a light pulse and the phase-shift it evokes is termed a phase–response curve (PRC) and was first described by DeCoursey 30 years ago in the flying squirrel [8]. Although a PRC to light has been measured in many mammalian and non-mammalian species [16], no such PRC has been demonstrated in humans. Indeed, it had been generally accepted that light was not as effective as social zeitgebers [23]. However, the discovery that high-intensity light (> 2500 lux) could suppress melatonin secretion in humans [17] re-aroused interest in the possibility of light as a zeitgeber. Several studies have now shown that bright light pulses over three or more successive cycles can phase-shift the human circadian timing system (for example refs. 3, 6, 9). These results cannot be interpreted as a PRC because of the use of multiple pulses under entrained conditions. We now report a classical PRC to a single light pulse. This is the first demonstration that the mechanism of entrainment in humans conforms to those in all other species studied.

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We carried out 19 experiments with 15 healthy student volunteers (age range 18–25 years), two of whom were women. They were studied individually under conditions of temporal isolation in our Isolation Unit, details of which have been described previously [12]. Their rectal temperature was automatically measured every 6 min. During the subjects' wakefulness the Unit was lit with standard tungsten lighting providing an intensity of 150–200 lux at about 1.7 m above ground level; at bedtime this was replaced by dim red light providing an intensity of less than 3 lux at bed level. Subjects spent most of their waking hours sedentary. For exposure to bright light, subjects sat in front of a single (5000 lux) or triple bank (9000 lux) of full-spectrum fluorescent lamps, and were required to look directly at the lights for 10 min in every 15; in between, they read with the book held so that their field of view was covered by the bright lights.

Protocol design was based on two of Aschoff's strategies for establishing a PRC [1]. In *Protocol 1*, 6 experiments were carried out using subjects initially entrained for 5 days to a 24-h clock to stabilise their circadian rhythms; the light pulse given at the end of this time fell early or late in the subjective night, analogous to times chosen for animal PRCs. The clock in the Unit was then removed and subjects were permitted to select times of sleep and wakefulness (free run) for a further 7–8 days. In *Protocol 2*, 10 experiments were performed in subjects allowed to free run from the outset. On day 7, at a time determined from their previous temperature cycles, they were exposed to a single light pulse and the free run continued for a further 7–10 days. *Protocol 3* was identical to the second except that bright light pulses were administered on three successive cycles. In accordance with

others [3, 4, 6, 7, 23] the body core temperature rhythm was used as a marker for the circadian pacemaker. Since activity increases and sleep decreases temperature, thus 'masking' any endogenous rhythm, accurate determination of circadian phase and amplitude requires that masking effects be removed. This can be done either by some form of constant routine [19] as was elaborated by Czeisler [4], or the effects of masking can be removed mathematically [13, 20, 24]. Because long periods of constant wakefulness, as demanded by the constant routine protocol, might perturb the clock and are inappropriate for free-running studies, we have chosen to use the second method. We have shown elsewhere that this method allows assessments of pacemaker phase that are superior to the use of masked data and are indistinguishable from results from constant routines [13, 20].

Phase shifts of the 'unmasked' temperature data were estimated in two ways. First, the periods of time when the rectal temperature fell below that day's mean were plotted in a raster format, and regression lines fitted to their onset (omitting the first post-light pulse day in case of transients). The two regression lines were then extrapolated to the day following the light pulse(s) to yield the phase shift. Two examples of the phase shifts derived from this method are shown in Fig. 1. The second method utilised the periodogram analysis [10] to assess the period of the rhythm, and the average waveform at this period was educed. The two educed waveforms (pre- and post-pulse) were cross-correlated, introducing a phase-lag progressively increased in steps of 0.1 h (the sampling frequency); the phase lag producing the highest positive cross-correlation estimated the phase shift (example in Fig. 3).

The phase-shifts estimated by the two methods were similar and not statistically different (by Student's paired t-test). They are listed in Table I for each protocol and averaged for pictorial representation in Fig. 2. A light pulse given before the rectal temperature minimum produced a phase delay, and after the minimum, a phase ad-

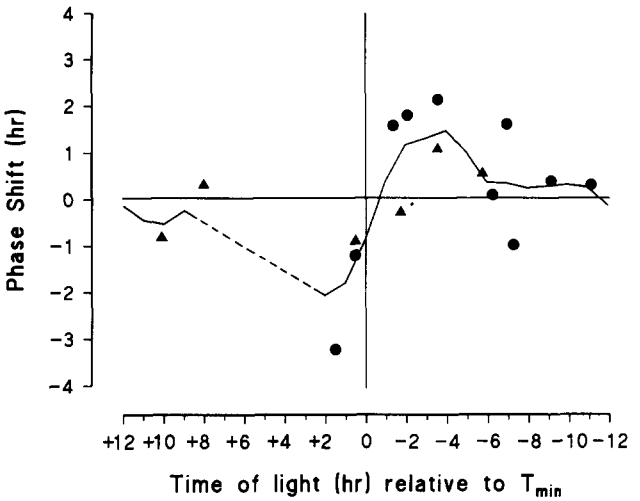

Fig. 2. Phase–response curve to single light pulses. Mean phase shifts, derived from the average of the two methods of estimation, are plotted as a function of time of the mid-point of the light pulse relative to the time of rectal temperature minimum. The mid-point of the light pulse is expressed as hours before (+), or after (−), the time of rectal temperature minimum. Negative phase shifts indicate phase delays. ▲: phase shifts derived from Protocol 1 experiments; ●: phase shifts derived from Protocol 2 experiments. The fitted curve is an hourly estimate of the phase shift using a weighted mean calculated from all values within the range of ±2 h. No estimate was made if less than two values were within this range (period of dashed line). Different weightings, with respect to overall bright and dim light exposure to estimate the mid-point of the light stimulus (see ref. 6), had little effect.

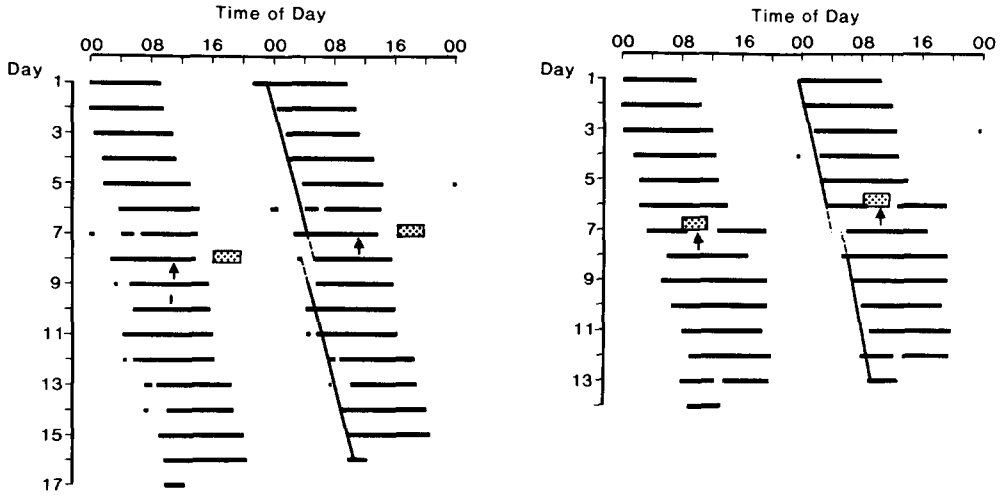

Fig. 1. Double plot of periods of time, horizontal bars, during which rectal temperature was below the daily mean, in two subjects performing Protocol 2. Data are plotted in raster format with successive days both next to and beneath each other. Diagonal lines: regression lines through onsets of these periods before and after the bright light pulse (indicated by stippled bar). The correlation coefficients for these regression lines were statistically significant ($P<0.05$). Dashed lines: extrapolation of these regression lines to the day following the light pulse. Estimated phase shifts were +1.41 h, left, and −1.73 h, right. Arrow-heads: time of temperature minimum on day of the light pulse.

TABLE I

PHASE SHIFTS PRODUCED BY LIGHT PULSES AT DIFFERENT TIMES

Phase shifts of the rectal temperature rhythm assessed from (a) regression lines through the times of onsets of temperatures below the daily mean (onsets) and (b) from cross-correlation of educed average waveforms (eduction): phase advance (+); phase delay (−). Time of light pulses expressed as time of mid-point of light pulse relative to the time of rectal temperature minimum: hours before temperature minimum (+); hours after temperature minimum (−). In protocol 3, time of mid-point refers to the first light pulse. Numbers in brackets indicate subjects studied on two occasions.

Protocol/ subject	Duration (h)/ intensity (lux) of light pulse	Time of light pulse relative to T_{min} (h)	Phase shift (h) assessed from	
			Onsets	Eduction
Protocol 1				
KAV	1/9000	+ 0.50	−1.00	−0.8
SMI	3/5000	− 5.80	−0.18	+1.3
COO {1}	3/5000	+10.10	−0.54	−1.1
COO {2}	3/5000	− 3.60	+0.25	+1.9
CLA {1}	3/5000	− 1.75	−0.17	−0.4
CLA {2}	3/5000	+ 8.00	−0.16	+0.8
Protocol 2				
WIL	3/9000	+ 0.50	−1.41	−1.0
GOW♀	3/9000	+ 1.50	−3.65	−2.8
LUK	3/9000	− 1.40	+2.17	+1.0
TUR {1}	3/9000	− 3.60	+1.56	+2.7
BOY	3/9000	− 6.30	+0.20	0.0
HAW	3/9000	− 7.30	+0.13	−2.1
RAN	3/9000	− 9.20	+0.50	+0.3
BYR♀	3/9000	− 2.10	–	+1.8
ASH {1}	3/9000	− 7.00	+1.73	+1.5
ASH {2}	3/9000	−11.20	+0.13	+0.3
Protocol 3				
BLI	3 × 3/9000	− 5.30	+5.40	+4.3
MUR	3 × 3/9000	− 0.40	+4.47	+4.0
TUR {2}	3 × 3/9000	− 0.10	+6.58	+7.1

vance. Although the first protocol did not result in as large and consistent phase shifts as did the second, a summary of all data indicate the existence of a human PRC to light (Fig. 2). It is to be noted that the phase advances of 1–2 h produced by a light pulse given about the time of habitual waking (normally 2–3 h after the temperature minimum) are of the order of magnitude required for daily exposure to outside light to entrain to the solar day a circadian pacemaker whose average endogenous period is 25 h [23].

Much larger phase shifts and a temporary diminution in amplitude of circadian rhythms have been obtained by others when entrained subjects were exposed to 5-h pulses of bright light given near the temperature minimum over three successive days [6, 15]. We have replicated such results under free-running conditions (see Table I, Fig. 3). The establishment of the phase-shifting effects of bright light provides a means of hastening the re-entrainment of the circadian pacemaker following time-zone transitions or shift work [5, 11]. Practical applications include circadian-related sleep disturbances [18, 22] or the treatment of winter depression [2], both of which have used 3 or more light pulses to enhance and stabilize this effect.

It is not possible to maintain human subjects in constant darkness for long periods as would be demanded using protocols classically used in other species to establish a PRC. In addition, the sleep–wake cycle, frequently used as a phase marker in other species, is less reliable in humans as can be seen in an initial study of the phase-shifting effects of light [14]. We have been able to show both phase advances and phase delays of the circadian temperature rhythm with single, bright light pulses in protocols close to those classically used. The human PRC does not differ from that of other species, except with respect to the light intensity required. The PRC is the basis of the mechanism by which entrainment to the solar day can be achieved, and is essential for temporal homeostasis.

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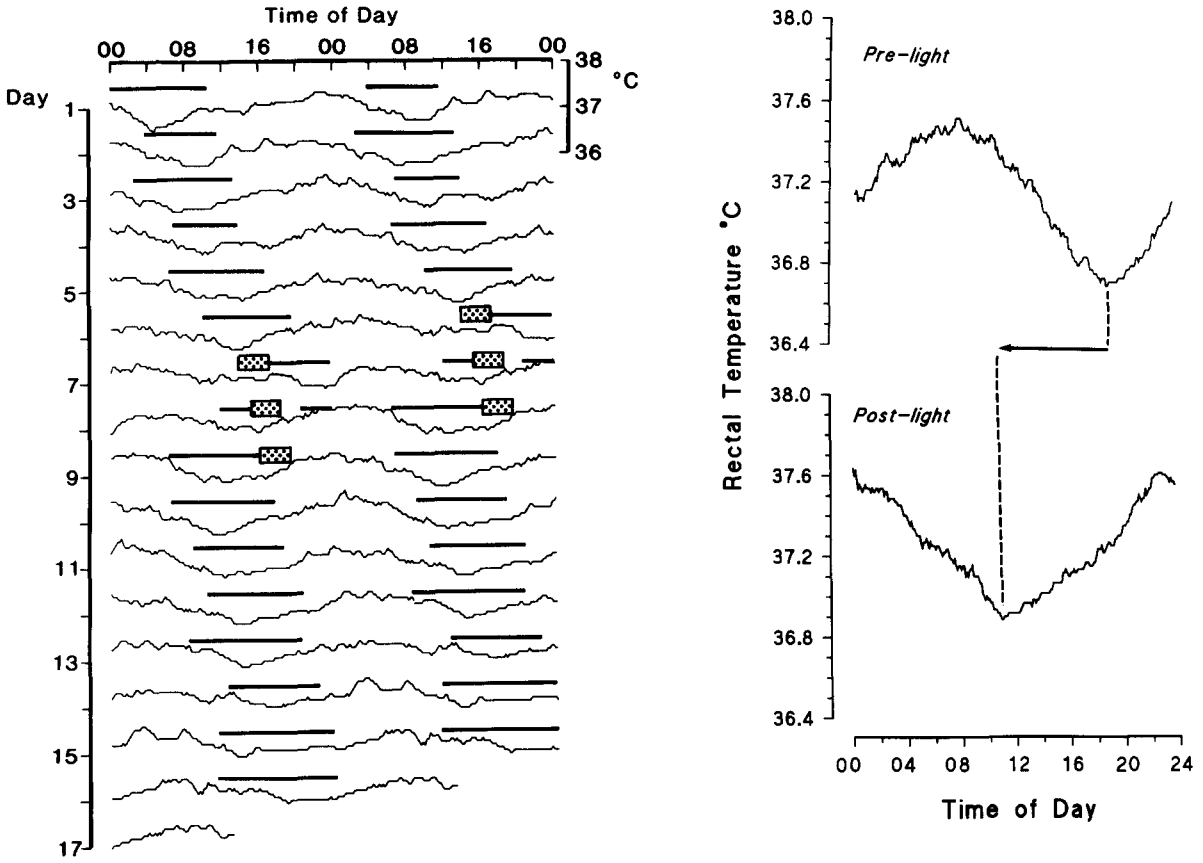

Fig. 3. Left: double plot of 'unmasked' rectal temperature rhythm in a single subject (TUR{2}) performing Protocol 3. Horizontal bars: periods of sleep; stippled bars: periods of bright light exposure. The amplitudes of the rhythm over days 7–9 (when bright light exposure took place) expressed as a percentage of the average amplitude of pre-light days (days 1–6), were 57.0%, 43.7%, 106.9%, respectively. The average amplitude over post-light days (days 10–16) was 102.7%. The decline in amplitude over the first two days of light exposure has been previously reported [6, 15]. Right: educed average waveforms of the data shown on the left, for the days before (*pre-light*) and after (*post-light*) the light pulses. Horizontal arrow (distance between vertical dashed lines) indicates size of phase shift (phase advance of 7.1 h) measured from cross-correlation of these average wave-forms.

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