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Light Intensity and Splitting in the Golden Hamster

GARY E. PICKARD,*[1] FRED W. TUREK† AND PATRICIA J. SOLLARS‡

**Department of Psychiatry, University of Pennsylvania, Philadelphia, PA 19104-6141, †Department of Neurobiology and Physiology, Northwestern University, Evanston, IL 60201, ‡Institute of Neuroscience, University of Oregon, Eugene, OR 97403*

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PICKARD, G. E., F. W. TUREK AND P. J. SOLLARS. *Light intensity and splitting in the golden hamster* PHYSIOL BEHAV **54**(1) 1–5, 1993.—Hamster circadian activity rhythms split into two components during prolonged exposure to conditions of constant light (LL). Several aspects of this phenomenon were examined in this study. The frequency of splitting was significantly greater among animals exposed to LL of 100 lux intensity (LL_{100}) compared with animals in LL_{10}. Animals that split had significantly longer free-running periods (τ) compared to nonsplitters and the decrease in τ associated with splitting was highly correlated with the presplit τ. Splitting was also observed under continuous dim light which fluctuated rhythmically from 5–10 lux. Thus, splitting of the circadian rhythm of activity is positively correlated with LL intensity with an LL intensity threshold for the induction of splitting in the range of 3–5 lux

Circadian rhythms Splitting Light intensity Hamsters

THE circadian activity rhythm can dissociate or split into two distinct components under constant lighting conditions (4,13). The splitting phenomenon is characterized by the two activity components temporarily expressing different free-running circadian periods until they stabilize approximately 180° out of phase. Attainment of the stabilized antiphase relationship between the activity bouts in the split state is almost always accompanied by a change in the period (τ) of the free-running rhythm compared to the period prior to splitting.

Although the physiological mechanisms underlying splitting of the circadian rhythm of locomotor activity remain largely unknown, ambient illumination intensity is an important factor related to the manifestation of splitting. Hoffmann (4) described splitting in the tree shrew (*Tupaia belangeri*), a diurnal mammal, as a function of ambient illumination intensity; the frequency of splitting increased as illumination intensity was gradually lowered to dim levels (1 lux). Recently, Meijer and colleagues (6) have reported similar splitting of the activity rhythm in tree shrews maintained in either dim LL (0.01–2.8 lux) or in constant darkness (DD). Splitting has also been reported in diurnal ground squirrels (*Eutamias sibiricus* and *Ammospermophilus leucurus*) maintained in dim LL and DD (11,14,15).

In contrast to diurnal mammals, splitting in the golden hamster, a nocturnal rodent, has been observed to occur only during exposure to LL conditions (5,13,16) and was generally assumed to be induced by bright light because Pittendrigh and Daan (13) did not observe splitting in light intensities lower than 100–200 lux. However, splitting of the hamster circadian rhythm of wheel running has recently been demonstrated to occur in LL_{50} and LL_{30} (3,5) but not in LL_3 (5).

Based primarily on the phenomenon of splitting, Pittendrigh and Daan (13) proposed a qualitative model of the circadian pacemaker regulating wheel-running activity as being comprised of two coupled oscillators. The quantitative model further developed by Daan and Berde (2) indicates that splitting may occur by either of two mechanisms related to changes in light intensity:

1. by opposite changes in the circadian period of the two coupled oscillators; or
2. by a change in the magnitude of the oscillators' influence on each other (coupling strength).

To further our understanding of the organization of the circadian pacemaker and the mechanisms underlying splitting we have examined several aspects of this phenomenon to:

1. determine whether the incidence of splitting is greater in LL_{100} compared to LL_{10};
2. examine the relationship between the presplit τ and the occurrence of splitting;
3. describe the correlation between the presplit τ and the decrease in τ associated with splitting; and
4. determine whether split activity rhythms develop under dim light fluctuating in intensity.

[1] Requests for reprints should be addressed to Gary E. Pickard, Ph.D., Department of Psychiatry, University of Pennsylvania, 422 Curie Blvd., Philadelphia, PA 19104-6141

METHOD

Male golden hamsters (*Mesocricetus auratus*) (8 weeks old, LAK.LVG, SYR, Charles River) were housed individually in cages equipped with running wheels with food and water freely available. Animals were maintained in light-tight, ventilated boxes, six cages to a box. Light was provided by a single 48-inch, 40 W fluorescent bulb, masked so that each cage received similar illumination (either 100 lux or 10 lux as measured at the floor of each cage). Wheel-running activity of each animal was monitored continuously throughout the experiment on an Esterline-Angus 20-channel event recorder as previously described (9).

Animals were initially maintained in a light:dark cycle (14 h light at 100 lux, 10 h dark at 0 lux) for 18–27 days. Following this initial period of entrainment, two groups of 48 animals/group were released into either LL_{10} or LL_{100} for 110 days. Data from two animals in the LL_{10} group were not included in the analysis, one animal became arhythmic and the other died.

Twenty-four additional animals were released into dim blue-green light (510 nm peak emission) emitted from electroluminescent lamps which rhythmically varied in intensity from a minimum of 5 lux to a maximum of 10 lux in the form of a sine wave with a period of 24 h; spectral characteristics were constant. After 45 days, the sinusoidal light cycle was phase delayed 6 h. On day 248, the sinusoidal light cycle was phase-delayed an additional 6 h. Twenty of twenty-four animals entrained to the fluctuating lighting conditions and their data have been previously reported (8).

Estimations of τ were made from the slope of an eye-fitted line drawn through 10 consecutive daily onsets of activity. To assess whether the occurrence of splitting is related to the period of the presplit rhythm, τ was estimated in LL_{10} and LL_{100} between days 25–35 (τ at 30 days) and days 55–65 (τ at 60 days) for all animals. The data were segregated into groups based on whether the animals activity rhythm split (split group) or remained intact or nonsplit throughout the study (intact group).

To assess the relationship between presplit τ and the change in τ upon splitting, the presplit τ was estimated from 10 days of activity onsets immediately prior to the occurrence of splitting. The period of the split rhythm was estimated beginning at the time the two bouts of activity demonstrated a stable phase relationship approximately 180° out of phase. In most cases, determination of the day splitting occurred was based on the identification of a bout of activity which dissociated from the main activity bout and free ran with a shorter period until establishing an antiphase relationship with the other activity bout. In the few cases when a transient free-running bout was not apparent before the split rhythm appeared, the first day a split pattern was apparent was used as the indicator of the occurrence of splitting.

Two-way analysis of variance (ANOVA) for unbalanced designs was used to examine the significance of differences between

1. the mean period of the intact (nonsplit) group vs the split group,
2. the mean period under LL_{10} vs. LL_{100}, and
3. whether the comparison between the intact and split groups depended upon light condition (LL_{10} vs LL_{100}). The 30 and 60 day data were examined separately.

RESULTS

Splitting in LL_{100} vs LL_{10}

Under LL_{100} conditions, the first onset of splitting was observed on day 27. The number of animals manifesting a split activity rhythm steadily increased until the experiment was terminated at about 110 days, by which time splitting was noted in 29/48 animals (60%, Fig. 1). Under LL_{10} conditions, the first onset of splitting occurred on day 37. Thirteen of the 14 animals that demonstrated splitting did so by day 82, 14/46 animals (30%) had split by the termination of the experiment (Fig. 1). The 60% incidence of splitting observed after 110 days in LL_{100} was significantly greater than the 30% incidence of splitting noted after 110 days in LL_{10} (Fisher's exact test, $p < 0.005$).

Relationship Between Presplit τ and Splitting

Housing hamsters under constant illumination produces both a lengthening in τ and splitting of circadian activity rhythms. After 30 days in LL, the two-way ANOVA model was significant, $F(3, 87) = 3.20$, $p = 0.03$, with the no interaction hypothesis accepted, $F(1, 87) = 0.45$, $p = 0.50$, indicating there was no evidence that the differences between intact and split groups were dependent on light intensity. In the no interaction model, the mean period of splitters was significantly greater than the mean period of animals which did not split (split 24.32 ± 0.03; $n = 37$ vs. intact 24.24 ± 0.02, $n = 54$), $F(1, 88) = 3.98$, $p = 0.049$.

After 60 days in LL, the two-way ANOVA model was again significant, $F(3, 68) = 8.79$, $p < 0.0001$. The interaction was statistically significant, $F(1, 68) = 4.02$, $p = 0.049$. Thus, there was some evidence that the difference in means between intact and split groups depended on light intensity. For LL_{10}, this difference was significant (split 24.70 ± 0.20; $n = 4$ vs intact 24.29 ± 0.03, $n = 32$), $F(1, 34) = 12.51$, $p = 0.001$, as was the difference for LL_{100} (split 24.55 ± 0.04, $n = 13$ vs intact 24.39 ± 0.04; $n = 23$), $F(1, 34) = 6.77$, $p = 0.01$. Thus, at both 30 and 60 days in LL, the mean (presplit) τ of splitters was greater than the mean τ of nonsplitters. Figure 2 illustrates the estimated period of each animal in LL_{10} and LL_{100} at both 30 and 60 days.

Table 1 summarizes the results for the mean τ of the intact and split groups in LL_{10} and LL_{100} after 30 and 60 days. An additional estimate of τ made between days 90–100 (τ at 95 days) of intact animals is provided in Table 1 and demonstrates further the steady increase in τ evident over time under both light intensities.

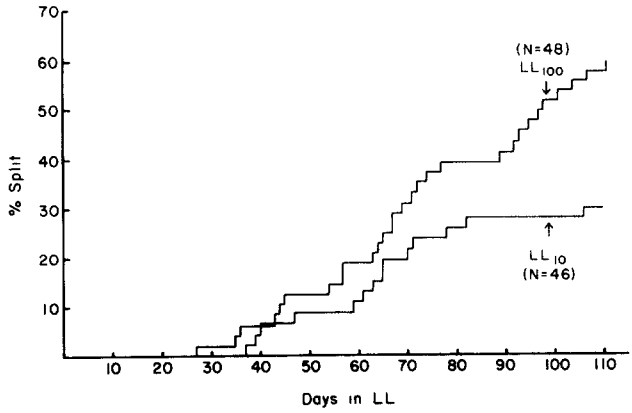

FIG. 1. The percentage of hamsters with split activity rhythms plotted as a function of days in constant illumination (LL). Sixty percent of the hamsters split under LL_{100} conditions, whereas 30% split under LL_{10} ($p < 0.005$).

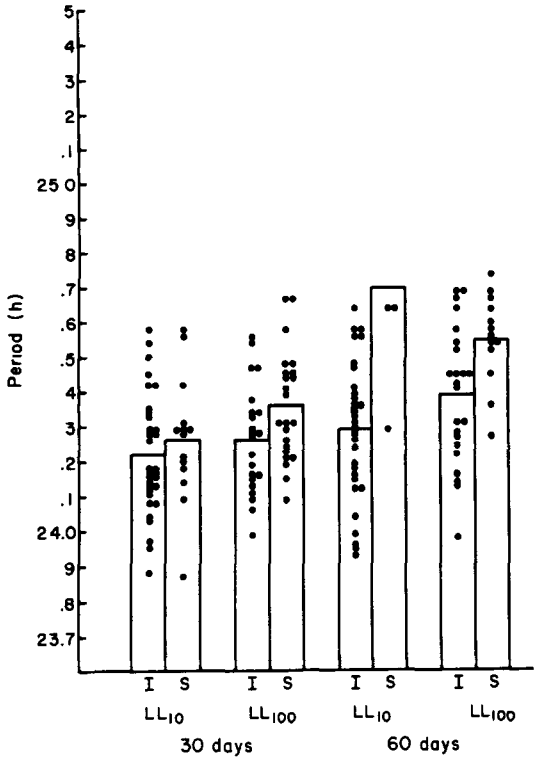

FIG 2 τ of animals that remained intact (I) (nonsplit) or that split (S) in LL_{10} and LL_{100} estimated at 30 days and 60 days in LL conditions. τ of individual animals and the group means are illustrated.

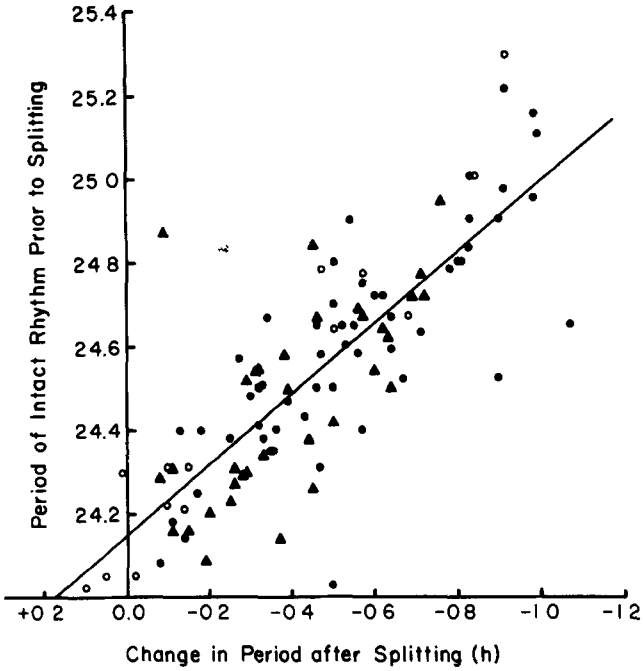

FIG. 3. Period of the intact or presplit circadian rhythm of wheel-running activity (estimated immediately prior to splitting) plotted as a function of the change in τ associated with the split activity rhythm [▲ = individual animals from this study, $n = 34$; ● = individual animals from previous studies, $n = 55$, see text; ○ = individual animals from Pittendrigh and Daan, (13) $n = 14$]. Composite regression line fitted to all data: $Y = 24.15 - 0.84X$, $n = 103$, $r = 0.81$).

Correlation Between Presplit τ and the Change in τ Upon Splitting

In the current experiment, the stabilization of the split activity components in an antiphase relationship was always accompanied by a decrease in τ. In 34/43 split animals, an accurate estimate of τ was made before and after splitting. The magnitude of the decrease in τ was positively related to the period of the circadian activity rhythm prior to splitting; the longer the free-running rhythm prior to splitting the greater the decrease in τ of the split rhythm ($r = 0.65$; regression line represented by $Y = 24.17 - 0.76X$; $n = 34$). This observation prompted the reanalysis of the change in τ associated with splitting from 55 animals involved in earlier studies (10,16). The positive correlation was again noted ($r = 0.80$) and the regression line for this data set was almost identical to the regression line describing the relationship in the present study ($Y = 24.16 - 0.81X$; $n = 55$).

After this correlation was determined, the data of Pittendrigh and Daan [p. 340; (13)] describing τ before and after splitting for 14 hamsters was compared with our results. Their data, described by the regression line $Y = 24.14 - 1.09X$ ($r = 0.96$; $n = 14$), include three animals which demonstrated a slight increase in τ upon splitting. The data of Pittendrigh and Daan are included in Fig. 3 The regression line plotted in Fig. 3 is a composite of the three data sets ($Y = 24.15 - 0.84X$; $r = 0.81$; $n = 103$).

Splitting in a Sinusoidal Light Cycle

Four of 24 animals failed to entrain to the sinusoidal light cycle varying in intensity from 5–10 lux; two demonstrated relative coordination (data not shown), and two developed a split activity rhythm. The wheel-running activity of the two splitters was similar and the activity record of one of the two splitters is presented in Fig. 4. Splitting of the circadian rhythm in each

TABLE 1

	30 Days		60 Days		95 Days
	Intact	Split	Intact	Split	Intact
LL_{10}	24.22 ± 0.03 ($n = 32$)	24.26 ± 0 05 ($n = 14$)	24.29 ± 0.03 ($n = 32$)	24.70 ± 0 20 ($n = 4$)	24.40 ± 0.04 ($n = 32$)
LL_{100}	24.26 ± 0 03 ($n = 22$)	24.36 ± 0.03 ($n = 23$)	24.39 ± 0.04 ($n = 23$)	24 55 ± 0 04 ($n = 13$)	24 51 ± 0.05 ($n = 23$)

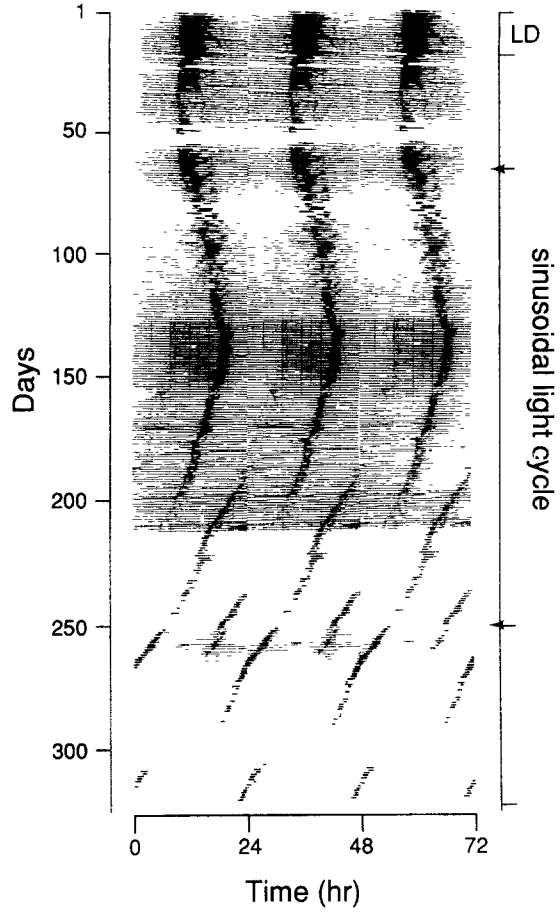

FIG 4 Wheel-running activity record of a hamster demonstrating splitting while maintained under a sinusoidal light cycle (see text). Each days activity record is presented beneath the previous days record and the data have been triple-plotted for clarity. LD = 14:10 light:dark cycle for days 1–17. Arrows indicate days on which the sinusoidal light cycle was phase delayed 6 h

animal occurred several weeks after a 6-h phase delay in the sinusoidal light cycle (Fig. 4)

A prominent feature of the activity record illustrated in Fig 4 is the reduction in wheel-running activity that occurred during the brighter fraction of the cycle. As each bout of activity free ran from the dim portion into the bright portion of the cycle, wheel running was greatly diminished, although still detectable Total activity increased again for each bout when the activity onsets drifted into the dimmer fraction of the light cycle. Masking of wheel-running activity was also clearly evident after the 6-h phase delay in the sinusoidal light cycle on day 248 (Fig. 4).

DISCUSSION

The occurrence of splitting of the hamster circadian activity rhythm in this study was a function of light intensity. Significantly more animals developed split activity rhythms in LL_{100} (29/48, 60%) than in LL_{10} (14/46; 30%). This observation is in good agreement with the recent work of McCormack (5) who showed that splitting occurred in 62% (8/13) of hamsters in LL_{400}, 43% (3/7) in LL_{30} and in no animals housed in LL_3 (0/15) or DD (0/19). These data, taken together, indicate that:

1 the frequency of splitting is positively correlated with LL intensity,
2 hamsters do split in relatively dim light with an LL intensity threshold for the induction of splitting in the range of 3–5 lux, and
3 the frequency of splitting in any cohort of hamsters approaches 60–70% in LL_{100} or greater [see also (16)]

One of the first descriptions of the splitting phenomenon also indicated that the occurrence of splitting was a function of ambient light intensity. However, Hoffmann (4) observed that the frequency of splitting of the circadian activity rhythm of the tree shrew increased as light intensity was decreased Thus, it might be concluded, based on the rather limited number of species which demonstrate splitting, that increasing light levels induce splitting in nocturnal mammals, whereas decreasing light levels induce splitting in diurnal animals

It is well known that τ in nocturnal rodents lengthens as a function of increasing LL intensity, whereas in many diurnal animals τ increases as LL levels decrease [Aschoff's Rule; (1)] Thus, both the frequency of splitting and the period of the free-running circadian rhythm in LL are related to LL intensity in a similar manner, as τ increases the frequency of splitting increases In this study, the mean τ of animals which demonstrated splitting was significantly greater than the mean τ of animals that did not split throughout the course of the study (Fig. 2) A similar relationship between long τs and the propensity to split was described by McCormack (5) Morin and Cummings (7) noted this relationship in gonadectomized hamsters treated with gonadal steroids but not in intact untreated animals

One of the essential features of the two coupled oscillator model of the mammalian circadian pacemaker developed by Pittendrigh and Daan is the dependence of τ on the phase relationship between the two oscillators, the increase in τ in LL is characterized by a change in the phase relationship between the coupled oscillators (2,13). The model also suggests only two stable phase relationships between the oscillators: either the oscillators are coupled inphase or 180° out of phase. Splitting represents the spontaneous shift from an inphase relationship to an antiphase relationship as the periods of the two oscillators are gradually forced apart under LL conditions (2) The steady state τ of the split rhythm is characterized by the reestablishment of a stable phase relationship between the oscillators in an antiphase position The period of the split rhythm with oscillators coupled in an antiphase relationship is not addressed in the model.

It was noted in this study that the magnitude of the decrease in τ observed at the onset of splitting is highly correlated with the presplit τ ($r = 0.81$, $n = 103$; Fig. 3). The mean τ of splitters in this study was 24.08 ± 0.03 ($n = 103$), the mean τ of splitters reported by Morin and Cummings (7) was similar (24.11 ± 0.02, $n = 35$) It is apparent that the period of the split rhythm is comparable to the species-typical τ of the golden hamster in DD [24.1 h, (12)]. If τ is dependent on the phase relationship between coupled oscillators, the data suggest that τ is comparable when the oscillators are either in phase (i.e., free-running rhythm in DD) or in an antiphase relationship (i.e., free-running split rhythm in LL)

It is of interest to note the masking of wheel-running activity that was prominent in the split animals maintained under sinusoidal lighting The small change in the amplitude of the light cycle (5 lux to 10 lux) was not great enough to entrain these individual animals, although 20/24 animals did entrain to these conditions (8) However, the small difference in illumination was clearly perceptible as indicated by the suppression of wheel-running activity during the brighter half of the light cycle. The

threshold for detecting a small change in light level sufficient to suppress locomotor activity is apparently lower than that required for entrainment, although the circadian system did respond to the different illumination intensities across the day by altering the period of the underlying oscillators. The functional dissection of the phenomena of entrainment, splitting, and masking by maintenance under sinusoidal light cycles might provide a valuable model to explore further the organization of the circadian system.

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