J. Comp. Physiol. 139, 49–58 (1980)

Journal
of Comparative
Physiology · A

Circadian Rhythms of Finches Under Bright Light: Is Self-Sustainment a Precondition for Circadian Rhythmicity?

Rütger A. Wever
Max-Planck-Institut für Verhaltensphysiologie, D-8131 Erling-Andechs, Federal Republic of Germany

Accepted May 20, 1980

Summary. Finches exposed to constant bright light of 50 and 500 lux, show continuous locomotor activity without any circadian rhythmicity. A rhythmic alternation of these intensities with a period of 24 h, however, can induce a similar activity rhythm. Some of the birds show a distinct rest-time while others which remain continuously active show fluctuations in the amount of activity. When the birds are synchronized by the same light intensities to 22 h, the phase relationship to the zeitgeber delays. This indicates that an oscillatory synchronization is present and that activity rhythms are not only passive reactions to changing light intensities. After the transfer from constant bright to constant dim light (1 lux), clear freerunning rhythms start immediately, with phases that are determined only by the instants of the transfer. All phenomena observed in the bright light experiments are in agreement with postulations of a simple oscillation model the validity of which had been shown previously.

The results of the experiments under bright light may have consequences in some different respects. For instance, they suggest that the evolutionary meaning of the circadian system is primarily the active reduction of the damping inherent in the biological processes; the endogenous generation of self-sustaining rhythms seems rather to be an epiphenomenon. An appendix deals with consequences with regard to the structure of the circadian system. The results of the former experiments with pinealectomized birds do not cogently implicit the assumption of a 'pacemaker' in the pineal organ. They are equally compatible with the assumption that the influence of the pineal organ on the circadian system is similar to that of light. A crucial experiment to discriminate between the two assumptions is proposed.

1. Introduction

Circadian rhythmicity is based on endogenous processes whose self-sustaining oscillations persist with periods slightly deviating from 24 h in the absence of any periodic input. In most species, the parameters of such a freerunning rhythm depend on experimental conditions, e.g. the intensity of illumination. Under the influence of the natural changes from day to night as well as that of artificial zeitgebers such as a light-dark cycle, the endogenously generated rhythm becomes externally synchronized. Consequently, the overt rhythms observed under normal conditions are considered a product of interactions between endogenous oscillations and external entraining signals.

It is also a well established fact that the capacity of circadian systems to produce self-sustaining oscillations is usually restricted to a certain range of conditions. This range is relatively small in comparison with the range of these conditions over 24 h in nature. In particular, circadian rhythms of many organisms can freerun only in continuous darkness or constant dim illumination. In continuous bright light of intensities which correspond to that of normal daylight, most organisms become arhythmic. Several avian species show freerunning activity rhythms in constant light up to about 10 lux. Below this, decreasing light intensities lengthen the period of the rhythm (Aschoff et al., 1962) and change, in addition, some other parameters (Aschoff and Wever, 1962b; Aschoff et al., 1971); above this threshold intensity, the birds are more or less continuously active. This seems somewhat contradictory to the fact that, in nature, the same birds behave rhythmically when exposed to light intensities several orders of magnitude above 10 lux during the day.

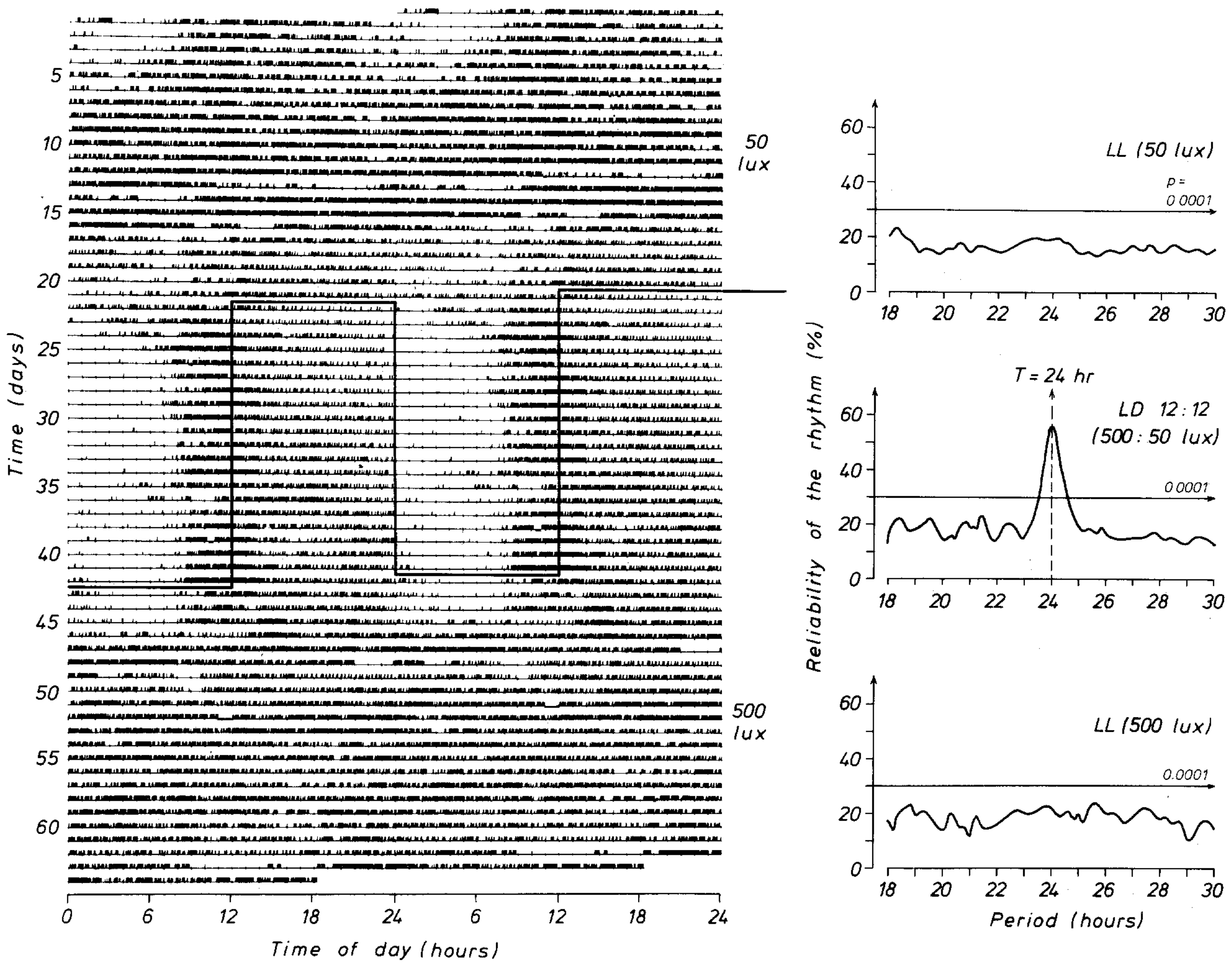

Fig. 1. Activity recordings from a brambling, kept in continuous bright light (*LL*) of 50 lux from day 1 to 21, in a 12:12 h *LD*-cycle alternating between 500 and 50 lux (with twilight transitions of 30 min each) from day 22 to 42, and in continuous bright light (*LL*) of 500 lux from day 43 to 63. Each horizontal line from 0 to 24 h represents the locomotor activity record of one day; records of successive days are plotted beneath each other. To facilitate the evaluation of the data, the records of two successive days are each plotted beside one another. In the right diagrams, period analyses are presented, computed from the activity data separately for the three sections of the experiment

In this paper data are presented from experiments in which birds have been kept under conditions of bright light with intensities well above the threshold for arhythmicity. The results are compared with those of mathematical computations based on an oscillator model. A final discussion concerns whether the capacity to produce self-sustaining oscillations in circadian systems, is of major relevance. An appendix should exemplify that the present considerations deduced from experiments under bright light may assist in evaluating physiological pathways in the circadian rhythm generation.

2. Methods

Six bramblings, *Fringilla montifringilla*, and 2 greenfinches, *Chloris chloris*, were singly caged in soundproof boxes. Each of the boxes was separately air-conditioned, and illuminated by incandescent bulb devices outside the boxes; overheating in the boxes, therefore, was largely avoided even under bright light conditions. The lamps were controlled by continuously variable, motor-driven dimmers. Illumination was either held constant for many days, or alternated between two different intensities, with varying periods and with twilight transitions of varying duration. Food and water were available ad libitum.

Locomotor activity of the birds was recorded by means of contacts under the perches connected to an event recorder and, simultaneously, a computer system which recorded the number of perch hoppings per minute. The latter was used as the basis for further analyses. Light intensity was recorded continuously by means of light sensitive resistors.

3. Results

After preliminary experiments had shown that the 8 birds used were continuously active in constant light

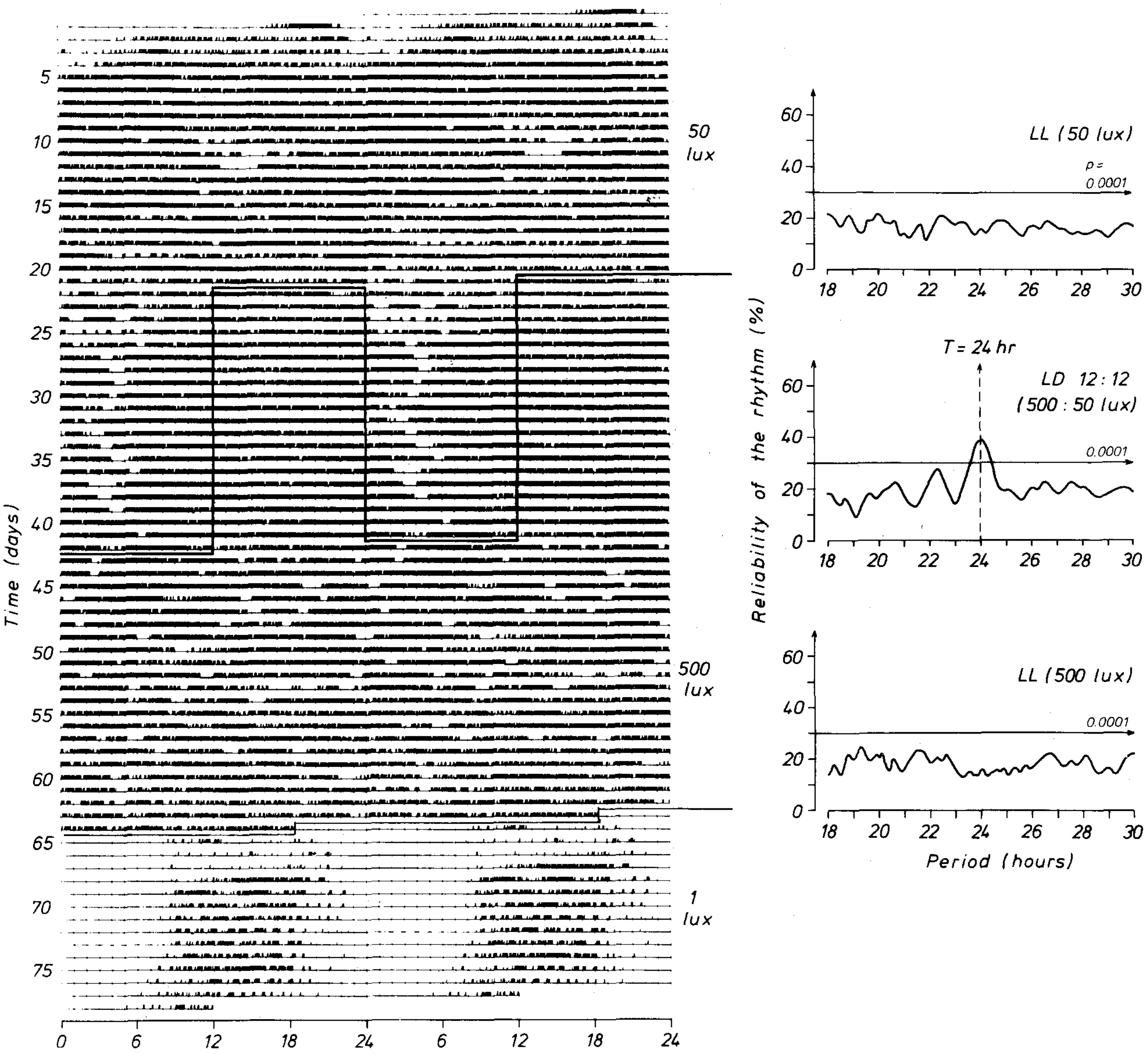

Fig. 2. Activity recordings from a brambling, kept for three weeks each in LL (50 lux), LD 12:12 h (500:50 lux), LL (500 lux) as the bird in Fig. 1, and subsequently in constant dim light of 1 lux. Designations as in Fig. 1. In the right diagrams, period analyses are presented, computed from the activity data separately for the first three sections of the experiment

above 20 to 30 lux, the experiments were carried out with intensities of 50 and 500 lux. The record of locomotor activity from a typical bird is reproduced in Fig. 1. The bird was exposed to constant light with an intensity of 50 lux for the first 21 days. The initial activity-rest cycle due to previous exposure to a light-dark cycle, faded away within a few days. The bird was then more or less continuously active, with only very short rest-times at irregular intervals. The period analysis (right diagram) did not reveal any rhythmicity within the circadian range. When exposed to alternating intensities of light (50 and 500 lux) the bird developed a clear activity rhythm in a few days which included a regular rest-time of several hours. The period analysis revealed a 24-h component in synchrony with the zeitgeber. In the third part of the experiment, the bird was exposed to 500 lux where it again became arhythmic in a few days. On the whole, the record demonstrates entrainment of the circadian system by alternating conditions each of which results in arhythmicity when provided continuously. Pure masking effects can be excluded by the positive phase-angle difference between onset of activity and the step from 50 to 500 lux. It is also worth mentioning that exposure to 50 lux does not unequivocally force the bird to be active because it can have a considerable rest-time in this intensity when entrained.

The results of a second bird (Fig. 2) differ from

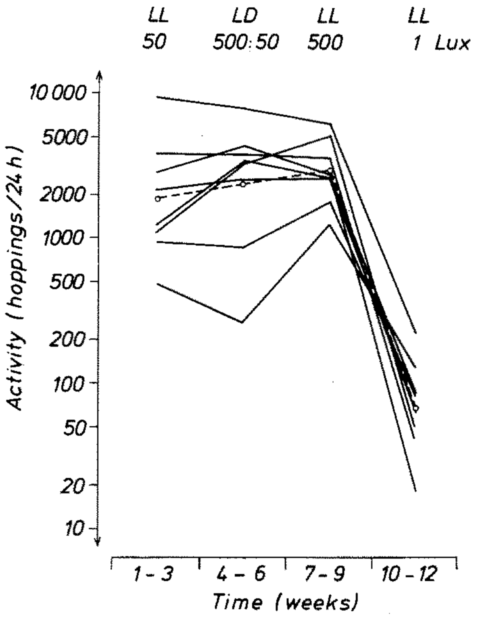

Fig. 3. Total amount of locomotor activity (perch hoppings) per 24 h (with logarithmic scale) from 8 finches (6 bramblings, 2 greenfinches), each averaged over a section of the experiment lasting 3 weeks. Data points of the 4 successive sections of each experiment (cf. Fig. 2) are combined by lines. Circles and dotted line: Means of all 8 birds

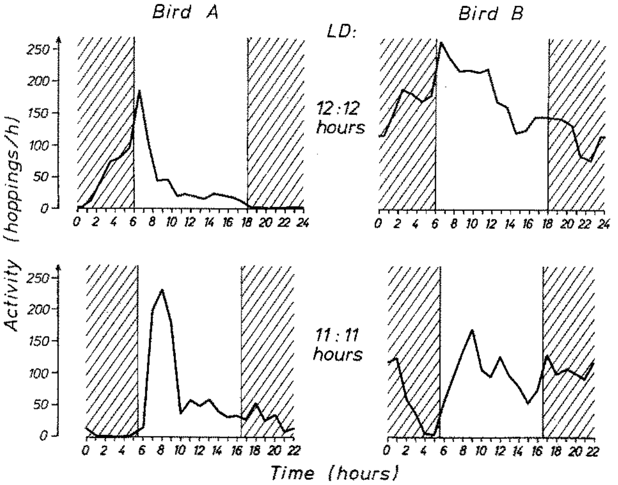

Fig. 4. Average activity cycles from 2 bramblings kept in LD-cycles alternating between 500 lux (open areas) and 50 lux (hatched areas; with twilight transitions of 30 min each). The periods are 24 h in the upper diagrams and 22 h in the lower diagrams. Bird A is the same as in Fig. 1, and bird B is the same as in Fig. 2. In each diagram, hourly data from 18 successive cycles are averaged (upper diagrams: 2nd sections of Figs. 1 and 2, after omission of the first 3 days each)

those above as the bird was continuously active during exposure to alternating light intensities. There were indications of short and irregular rest-times in 50 lux. Nevertheless, the period analysis again reveals a reliable 24-h component, in contrast to full arhythmicity during the first and third section of the experiment (Fig. 2, right diagrams). To demonstrate that the high level of activity was due to the specific conditions of bright light, the actogram includes the fourth section where the bird was exposed to constant dim light (1 lux). Immediately after the transfer, a freerunning rhythm emerged with a circadian period of 23.8 h. The experiment demonstrates that an actogram with seemingly continuous activity should not necessarily be interpreted as having no rhythmicity at all (cf. sect. 2). It can further be concluded that a bird with no rhythmicity in bright light, still has an intact 'clock' which can be entrained but which cannot manifest itself in an overt activity rhythm.

Results similar to those described were obtained from 6 other birds: arhythmicity in constant bright light of 50 and 500 lux, and entrainment to 24 h in alternating 50 and 500 lux. In constant dim light of 1 lux, all birds showed freerunning rhythms with periods ranging from 23.7 to 25.1 h. The transition from 500 to 1 lux was always accompanied by a drastic reduction in the amount of activity. In Fig. 3, the mean levels of locomotor activity during the four experimental sections (cf. Fig. 2) are drawn for each of the 8 birds. Apart from interindividual differences in level and minor variations between the first three sections (the average activity level slightly increased with increasing light intensity), activity was consistently high in bright light, and was reduced on the average by a factor of 40 during the exposure to 1 lux.

Two objections could be raised against the conclusions drawn from Figs. 1 and 2. One concerns the interpretation that the records obtained in section 2 represent true entrainment. This assumes that the alternation between activity and rest (Fig. 1) or high and low activity values (Fig. 2) is of an oscillatory origin, instead of assuming a passive reaction to changing light intensities. In Fig. 1, the oscillatory origin is obvious from the leading phase of the activity rhythm. Moreover, in this experiment the distinct rest-time suggests an oscillatory origin of the synchronization: A purely passive reaction would have produced the same amount of activity during the 12 h of lower light intensity as had been observed during the constant light section at this intensity. In Fig. 2, the assumption of an only passive reaction cannot be rejected as easily. Hence, a second test for the mechanism of synchronization was added. In these experiments, the birds were exposed to an alternation of 50 and 500 lux with a period of 22 h (LD 11:11) instead of 24 h (LD 12:12; including twilight transitions of 30 min duration in both cases). All birds remained synchronized to the 22-h zeitgeber as they had to the 24-h zeitgeber; and the phase of the activity rhythm were clearly later relative to the 22-h than to the 24-h zeitgeber. This is demonstrated in Fig. 4 by the activity patterns of the two birds whose actograms are reproduced in Figs. 1 and 2. In the 24-h day, both birds have a maximum of activity around 'sun-rise' and a minimum during the first half of 'dark-time'; in the 22-h day, the maxima occur several hours after 'sun-rise' and the minima during the second half of 'dark-time'. The results from the other six birds are similar. In summary: the activity rhythm was delayed in the short zeitgeber period compared with the long one. This result is in full agreement with an oscillatory origin of synchronization, and it disproves the assumption of a passive reaction to light as the cause for the regular fluctuations in locomotor activity under the influence of regularly alternating light intensities.

The second possible objection concerns the conclusion that, in constant bright light, there was no rhythmicity at all. It could be argued that there was, in fact, a maintaining rhythmicity, but that this rhythmicity was not manifested in an overt activity rhythm. The validity of this argument can be tested. After the long-term exposure to bright light, all birds were transferred to constant dim light of 1 lux intensity, but at varied times of day. All birds immediately started to show a freerunning rhythm (cf. Fig. 2). If this rhythm had been the continuation of a rhythm that was only obscured in bright light, the phase of the rhythm appearing in dim light should be influenced, at least in part, by the previous phase; if, on the other hand, there was really no rhythmicity in bright light and the rhythm started only with the transition to dim light, the instant of this transition should exclusively determine the later phase. In the experiment shown in Fig. 2, the transition from bright to dim light was at 18:00 h CET, and the first onset of activity in the later freerunning rhythm extrapolated to 8:30 h, i.e., it occurred 14.5 h after the transition. In the other experiments, the transition from bright to dim light was shifted to other times of day in regular intervals. Figure 5 shows the phases of activity onset in dim light from all 8 experiments as a function of the transition time from bright to dim light. There was a constant time lag between both instants of, on average, 13.6 h. This means, the phase of the freerunning rhythm was determined exclusively by the transition from bright to dim light, and there was no hint of an additional influence from some hypothetically persisting rhythm which was obscured by bright light. Similar results have been obtained by Binkley (1978). Together, these findings indicate that a previously arhythmic system started to become rhythmic with the transition from bright to dim light. It is simply another way of saying that a completely desynchronized system consisting of many steadily running oscillators became internally synchronized by the transition from bright to dim light.

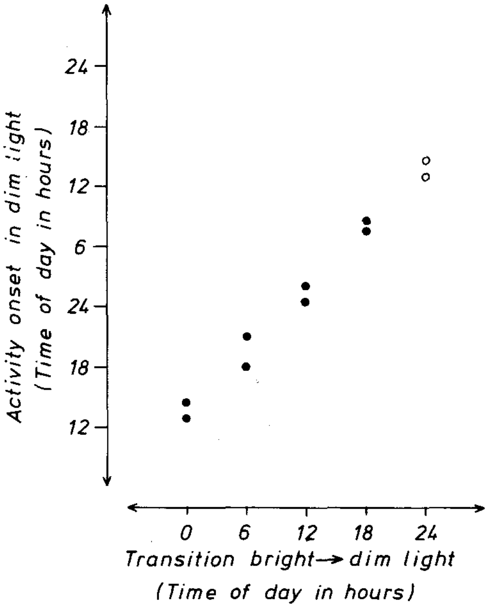

Fig. 5. Phase of the activity rhythm freerunning in constant dim light, plotted as a function of the phase of the transition from constant bright to constant dim light, extrapolated each to the first activity onset following the transition (Fig. 2). Data from 8 finches. Open circles at 24 h: Redrawings of data points from 0 h

4. Theoretical Considerations

The results obtained in the bird experiments are in good agreement with postulations of a simple mathematical model of circadian rhythms (modified van der Pol equation; Wever, 1964, 1965, 1966). This means that peculiarities of circadian rhythms to be observed under certain conditions, are nothing but consequences of simple oscillation laws; the applicability of these laws to biological systems had been shown previously. It may, therefore, facilitate the understanding of circadian phenomena under bright light when solutions of the model oscillation under corresponding conditions are considered.

Every system capable of generating self-sustaining oscillations is characterized by a feed-back mechanism restoring the energy that is inevitably dissipated by 'friction'. In steady state, energy output and input neutralize each other, so that the 'net damping' is, on average, zero. To guarantee stability, the 'net damping' cannot be temporally constant within one cycle. It must rather vary with the elevation of the system from its neutral position. The 'net damping', therefore, increases not only with the amplitude but also with increasing deviation of the mean value from the neutral position. It is for this reason that the feed-back mechanism is only able to neutralize the 'friction' completely within a limited 'oscillatory range' of mean values (Wever, 1963). Every oscillator is capable of generating self-sustaining oscillations only as long as the 'external force' holds its mean value inside a certain range. Outside this 'oscillatory range', the feed-back mechanism is still in operation as inside this range; it reduces the 'net damping', but it is no longer sufficient to neutralize the 'friction' completely. As a result, the oscillation fades away after any push. Here, the system works like a 'narrow bandwidth amplifier', or a 'resonance amplifier', which only amplifies stimuli that have periods in a small range around 24 h. It is only with a much greater elevation of the system that the feed-back mechanism is eventually not even sufficient to guarantee oscillatory synchronization to rhythmic stimuli. Outside the 'range of periodic adaptation', the system reacts aperiodically to changing conditions (Wever, 1963).

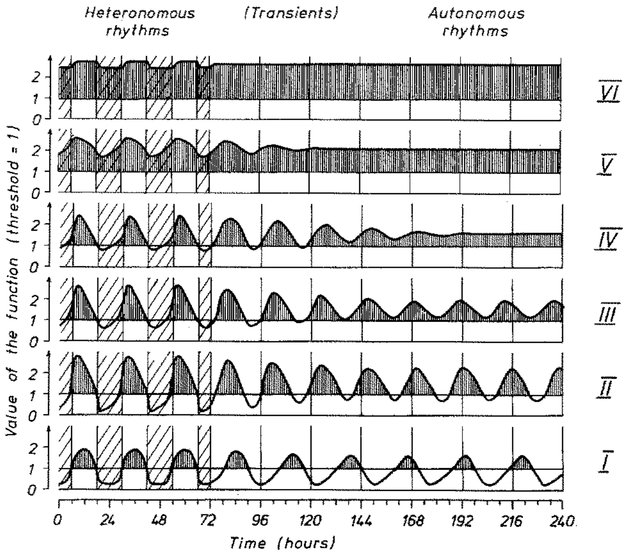

Fig. 6. Six solutions of a mathematical model of circadian rhythms (Wever, 1964, 1965, 1966): $\ddot{y}+0.5(y^2+y^{-2}-3)\,\dot{y}+(0.6\,y+1)\,y=\ddot{x}+\dot{x}+x$, computed with six different 'external forces' (I: $x=1.6$; II: $x=3.0$; III: $x=3.15$; IV: $x=3.3$; V: $x=5.0$; VI: $x=7.0$), corresponding to (from I to VI) increasing light intensity. With each 'light intensity', at first solutions under the influence of 3 successive 'LD-cycles' (12:12 h; with $\Delta x=\pm 0.5$ in all cases) are presented; after 72 h, 'light intensity' is held constant but, after fading away transients of different durations, only the last few days represent the steady state under constant conditions. The hatched areas above the threshold represent 'activity' and the open areas below the threshold (if present) represent 'rest'

To illustrate the behavior mentioned, Fig. 6 presents six solutions of the model equation, computed with different 'external forces' (right side of the differential equation). When applied to circadian rhythmicity, the 'external force' is analogue to the external condition controlling the rhythm. In this example, it corresponds to light intensity. In the diagrams of Fig. 6, the 'external forces' increase from I to VI; they are selected to show typical solutions.

From the six diagrams presented in Fig. 6, only diagrams I and II cover the range where circadian experiments usually are performed. Under constant conditions (right), the oscillation is self-sustaining with a clear separation between activity (above the threshold) and rest (below the threshold). With increasing 'light intensity' (I→II), the period of the freerunning rhythm shortens. The amplitude increases as well as the total amount of activity per unit time and the ratio between activity- and rest-time. Moreover, the shape of the rhythm changes typically from 'skewed to the right' to 'skewed to the left', and the variability of the rhythm under the influence of a certain 'noise level' decreases. Under the influence of a zeitgeber (left), the rhythm stays synchronized. Apart from changes in amplitude and shape, the phase of the rhythm advances, with the transition from I to II, relative to the zeitgeber. In condition III, there is, under constant conditions, still a freerunning rhythm. The rhythm, however, no longer falls below the threshold separating activity and rest, so that it describes 'continuous activity' (with rhythmically alternating amounts of activity). In comparison to condition II, the amplitude of the rhythm is decreased, the shape is more symmetric, and the variability under the influence of the 'standard noise' is increased. Under the influence of the zeitgeber, there is a synchronized rhythm with clear separation between activity and rest; and the phase of the rhythm is further advanced relative to the zeitgeber. After removing the zeitgeber, there are some transient cycles still showing coherent rest-times until the amplitude has decreased to its steady state value and continuous activity appears. In summary, the diagrams I to III describe the behavior of the oscillation within the 'oscillatory range'; and this theoretically deduced behavior is in full agreement with experimental findings (Aschoff et al., 1971; Wever, 1971).

In diagram IV, the limit of the 'oscillatory range' has been exceeded. Under constant conditions (right), there is continuous and constant activity; the oscillation is no longer self-sustaining, it behaves rather like a damped oscillation. Under the influence of the environmental cycle (left), there is a synchronized rhythm with separated activity and rest, like in the previous diagrams. The phase of the rhythm is still more advanced relative to the zeitgeber than in case of diagram III. Although there is a fundamental difference in the behavior of the system inside and outside the 'oscillatory range' as long as the external conditions are temporally constant (right part of Fig. 6), there are continuous changes in the rhythm parameters under periodically alternating conditions (left part of Fig. 6). In other words: the limit of the 'oscillatory range', or the distinction between self-sustaining and non self-sustaining oscillations respectively, is, indeed, relevant under constant conditions, but has no meaning under the influence of an external zeitgeber of sufficient strength. Compared with the animal experiments discussed in this paper, diagram IV seems to be a good model for the rhythmic behavior of some of the birds under bright light (cf. Fig. 1). If the 'external force' increases further (diagram V), not only the mean level increases but mainly the amount of the 'net damping'. Under constant conditions, the system oscillates as little as in diagram IV. Under the influence of the synchronizing environmental cycle, the amplitude is smaller due to the increased net damping. The rhythm remains above the threshold indicating continuous activity although the amount fluctuates. Diagram V seems to be a good model to describe the rhythmic behavior of other birds in bright light (cf. Fig. 2). It should be noted that, under all 'external forces' discussed so far (diagrams I to V), the phase relationship of the synchronized oscillation to the environmental cycle depends on the period of this cycle: with shortening period, the oscillation is delayed relative to the environmental cycle.

With increased 'external force' (diagram VI), the 'range of periodic adaptation' is eventually left, and the system behaves aperiodic. This means, it reacts only passively to changes in environmental conditions, without any temporal dependence. In this condition of aperiodic adaptation, the amounts of activity depend on the actual intensity of illumination, in periodically alternating conditions with the same relation as in constant conditions. A change in activity always follows the change in light intensity with a constant time lag; it follows a single light step as well as rhythmic alternations of any period. The limit of the 'oscillatory range' (at $x = 3.189$; between diagrams III and IV) is the turning-point in the behavior of the system under constant conditions, whereas the limit of the larger 'range of periodic adaptation' (at $x = 6.730$; between diagrams V and VI) is the turning-point under the influence of periodically alternating conditions. Inside the latter range, forced oscillations under the influence of zeitgebers are present. The properties of these oscillations are widely independent of the self-sustainment capacity. Outside this range, there are only passive reactions to external stimuli. On the other hand, the limit of the 'range of periodic adaptation' is irrelevant under constant conditions, since there is continuous activity without any rhythmicity below as well as above this limit.

5. Conclusions

The main results of the experiments performed with finches under bright light can be summarized in two statements:

1. An alternation between two conditions under which when used alone produces no circadian rhythmicity, potentially acts as a zeitgeber forcing a synchronized rhythm.
2. The absence of the self-sustaining capacity under certain conditions need not lead to the conclusion that there is no intact 'clock mechanism'.

Both these experimental findings are in agreement with theoretical postulations of a simple model describing self-sustaining oscillations. It is concluded that there are different ranges of rhythmic behavior, depending on external conditions. Within a narrow 'oscillatory range', the system can produce self-sustaining oscillations, and within a larger 'range of periodic adaptation', the system can be forced to oscillate by external periodicities. The rhythmic behavior is determined by the 'net damping' of the system which depends on the elevation of the system from its neutral position. Within the 'oscillatory range', an active feed-back mechanism neutralizes the inevitable 'friction' of the biological processes to a zero net damping; and within the 'range of periodic adaptation', the feed-back mechanism reduces the net damping down to an amount guaranteeing oscillatory synchronization but is not always sufficient to neutralize the friction completely.

Putting together experimental and theoretical results, it has to be concluded that the feed-back mechanism, i.e. the 'clock', is of adaptive value even when it does not lead to self-sustaining rhythms. It reduces, in any case, the damping of the system. In dim light, the active clock mechanism is sufficient to neutralize the inevitable friction completely; the result is a self-sustaining rhythm in constant conditions, and a synchronized rhythm in periodically changing conditions. In bright light which corresponds to natural daylight intensities, the influence of the friction on the damping superceeds that of the clock mechanism. The result is a rhythm with a still varying net damping, oscillating not around zero as in self-sustainment but around a positive level. In constant conditions, this rhythm is non self-sustaining but fades away. Periodically changing conditions are capable of synchronizing this rhythm similar to the rhythm in dim light. Although self-sustaining and non self-sustaining rhythms are very easily distinguishable in constant conditions, they cannot be distinguished from one another under the influence of a synchronizing zeitgeber. This is true as long as the period of the zeitgeber does not differ too much from the natural period of the rhythm, i.e., if it is within the range of entrainment of the self-sustaining rhythm. In particular, phase and amplitude of the rhythm depend on the ratio between zeitgeber period and natural period, nearly identically in both self-sustaining and non self-sustaining rhythms. On the other hand, without the intact clock mechanism in operation, the remaining friction would hinder synchronization to a zeitgeber especially in bright light; it would only allow a passive, aperiodic reaction to alternating environmental conditions.

Many birds in nature show a leading phase relationship to the day-night cycle in their activity rhythms (Aschoff and Wever, 1962a). Such a phase relationship cannot be produced by a system which only passively reacts to the alternation between light and dark. It is, rather, always a consequence of oscillatory synchronization. It is, however, not bound to self-sustainment of the rhythm but is applicable also for systems only capable of damped oscillations. With regard to the temporal stability of such a system, it seems advantageous to have a large circadian amplitude (Wever, 1979, 1980). Only in an oscillatory system under the influence of environmental alternations with a period close to the natural period of the system would the rhythm be amplified, resulting in an enlarged amplitude. In a passively reacting system, the different states of the system reflect only the different environmental conditions, independent of the temporal pattern.

In line with these considerations, it may be speculated that the biological meaning of the 'clock mechanism' is, apart from being able to oscillate with a period close to 24 h, to reduce actively the net damping of the circadian system. A small net damping enables oscillatory synchronization but not necessarily self-sustainment. In fact, a remarkably small overall damping is to be deduced from observations of the existing circadian systems under all conditions, e.g. in bright as in dim light. It might be an overstatement of facts to say that self-sustainment is an artifact present only within a small range of artificial conditions which has no important meaning in nature. However, one must reckon with the fact that the reduction in the overall damping of the oscillatory system is performed by the 'clock mechanism' under all conditions even when self-sustainment is not present. This decrease in the overall damping which leads to oscillatory synchronization fulfills the biological meaning of the circadian system completely. It might then be appropriate to describe the 'clock' as a mechanism for reducing the damping inherent in the circadian system. Under special conditions, this mechanism could produce self-sustaining rhythms as an epiphenomenon.

6. Appendix

The conclusions of the experiments performed under bright light may be applicable to other conditions where organisms show continuous activity without recognizable rhythmicity. Especially the second of the two summarizing results mentioned above deserves attention: it can be wrong to conclude from the absence of a self-sustaining capacity under certain conditions to the absence of an intact 'clock mechanism'. Consequently, the consideration of these results may assist in the critical examination of experiments aiming at the evaluation of physiological pathways in the generation of circadian rhythms. As an example, in the following observations after pinealectomy of birds will be discussed.

Gaston and Menaker (1968), and Menaker and Zimmerman (1976) have shown that, after pinealectomy, sparrows are continuously active even in constant darkness. The authors conclude that, by pinealectomy, the 'master clock', or the 'pacemaker' of the circadian system has been removed. The same authors have, in addition, shown that the pinealectomized sparrows can be synchronized to 24 h by a light-dark cycle. They propose the existence of an auxiliary clock outside the pineal which is likewise sensitive to light. This clock is not capable of self-sustaining oscillations but only of damped oscillations. It is normally driven by the 'pineal clock', and it directly controls the locomotor activity of the birds. Gwinner (1978) reported similar results in starlings. His birds, although likewise continuously active after pinealectomy, often showed a slightly persisting rhythm. He concluded that several auxiliary clocks exist which are also capable of self-sustaining oscillations with periods shorter than that of the 'pineal clock'. In this modified model, all auxiliary clocks are synchronously driven by the master clock as long as the pineal organ is intact. After pinealectomy, the auxiliary clocks persist autonomously, resulting in either an overt rhythm when mutually coupled, as in the starlings, or a damped rhythm when mutually desynchronized, as in the sparrows.

The results obtained from birds after pinealectomy under constant darkness correspond extensively with the results obtained from intact birds under bright light (cf. Figs. 1 and 2). If representing the same phenomena, the light simulated by pinealectomy would be brighter in sparrows than in starlings, corresponding to the difference between diagrams IV and III in Fig. 6. Gaston and Menaker (1968) have already discussed this analogy. They rejected this interpretation since "it appears unlikely in view of the fact that pinealectomized birds can be entrained by light cycles" (Gaston and Menaker, 1968). In view of the present experimental results (cf. Fig. 1) as well as the theoretical considerations (cf. Fig. 6), this rejection is not tenable. The results obtained with pinealectomized birds are, therefore, compatible with both, the 'master clock', or 'pacemaker' hypothesis as well as with the hypothesis that the clock mechanism is outside the pineal and is pushed out of the oscillatory range by pinealectomy, just as it is pushed by the transfer to bright light. Pinealectomy then raises the overall damping of the circadian system; it does not change the operational mode of the 'clock', just as little as it does the transfer to bright light.

In the attempt to discriminate between both hypotheses, one might refer to the effects of a pineal gland that is transplanted from a donor bird into the anterior chamber of the eye of a pinealectomized bird. After transplantation, the previously continuously active and arhythmic recipient bird becomes rhythmic (Zimmerman and Menaker, 1975), and the freerunning rhythm assumes the previous phase of the donor bird (Zimmerman and Menaker, 1979). It has been concluded that, with the transplantation of the pineal gland, not only the master clock itself had been transferred but also the special phase of the running clock. There are, however, reasons to think that the result of this transplantation experiment is likewise compatible with the other hypothesis. If pinealectomy corresponds to the transfer to bright light, the implantation of an intact pineal corresponds to the return into dim light (4th section of Fig. 2). It had been shown that the phase of the rhythm which starts to freerun after this transfer, is determined by the instant of this transfer (cf. Fig. 5). It is, therefore, the relevant question what instant after the transplantation is analogue to the instant of the transfer from bright to dim light, i.e., what instant starts the rhythm. It had been shown that rhythmic injections of melatonin in a pinealectomized bird can establish a rhythm whose phase is determined by the instants of the injections (Gwinner and Benzinger, 1978). It has also been shown that extirpated and isolated pineal glands have the capacity to continue with rhythmic enzyme activities (Binkley et al., 1977; Kasal et al., 1979), as other organ cultures can continue to show circadian patterns in metabolism. Seen together, these findings suggest that it is not the instant of the transplantation of the pineal itself that starts the rhythm in the recipient bird, but the instant of the first melatonin excretion or the rhythmically repeated instants of excretion from the transplanted gland. This instant, however, is clearly determined by the previous phase of the donor bird. Following these arguments, the results of the transplantation experiments are in full agreement not only with the first hypothesis (pineal as the 'pacemaker') but also with the other hypothesis. They correspond to the results demonstrated in Fig. 5. There are some secondary results apparently supporting the first hypothesis; the understanding of these results, however, needs additional assumptions with either hypothesis. For instance, the effect of continuous melatonin administration in intact birds under constant darkness may be mentioned, which either shortens the freerunning period or induces continuous activity (Turek et al., 1976).

The discrimination between the two hypotheses is perhaps possible by performing an experiment in which low doses of melatonin are continuously administered to a pinealectomized bird in constant darkness. Without this administration, the bird should be continuously active without any circadian rhythmicity. If the first hypothesis is correct and the 'pacemaker' is located in the pineal organ, the continuous administration of melatonin should not essentially change the picture; in particular, it should not induce a freerunning rhythm. If, on the other hand, the second hypothesis is correct and the pineal organ assists in reducing the damping of the circadian system which is located somewhere else, the continuous administration of melatonin should correspond to the transfer from bright to constant dim light, and a freerunning rhythm should appear. This experiment, of course, is crucial only in discriminating between the two hypotheses mentioned concerning the meaning of the pineal organ in the generation of circadian rhythms in birds; it is without relevance with regard to the meaning of self-sustainment of circadian rhythms as deduced from experiments in bright light which is the topic of this paper.

References

Aschoff, J., Wever, R.: Beginn and Ende der täglichen Aktivität freilebender Vögel. J. Ornithol. **102**, 2–27 (1962a)

Aschoff, J., Wever, R.: Aktivitätsmenge and $\alpha{:}\rho$-Verhältnis als Meßgrößen der Tagesperiodik. Z. Vergl. Physiol. **46**, 88–101 (1962b)

Aschoff, J., Diehl, I., Gerecke, U., Wever, R.: Aktivitätsperiodik von Buchfinken (*Fringilla coelebs* L.) unter konstanten Bedingungen. Z. Vergl. Physiol. **45**, 605–617 (1962)

Aschoff, J., Gerecke, U., Kureck, A., Pohl, H., Rieger, P., Saint Paul, U. von, Wever, R.: Interdependent parameters of circadian activity rhythms in birds and man. In: Biochronometry. Menaker, M. (ed.), pp. 3–27. Washington D.C.: Natl. Acad. Sci. 1971

Binkley, S.: Light-to-dark transition and dark-time sensitivity: importance for the biological clock of the house sparrow. Physiol. Zool. **51**, 272–278 (1978)

Binkley, S., Riebman, J.B., Reilly, K.B.: Time keeping by the pineal gland. Science **197**, 1181–1183 (1977)

Gaston, S., Menaker, M.: Pineal function: the biological clock in the sparrow? Science **160**, 1125–1127 (1968)

Gwinner, E.: Effects of pinealectomy on circadian locomotor activity rhythms in European starlings, *Sturnus vulgaris*. J. Comp. Physiol. **126**, 123–129 (1978)

Gwinner, E., Benzinger, I.: Synchronization of a circadian rhythm in pinealectomized European starlings by daily injections of melatonin. J. Comp. Physiol. **127**, 209–213 (1978)

Kasal, C.A., Menaker, M., Perez-Polo, J.R.: Circadian clock in culture: N-acetyltransferase activity of chick pineal glands oscillates in vitro. Science **203**, 656–568 (1979)

Menaker, M., Zimmerman, N.H.: Role of the pineal in the circadian system of birds. Am. Zool. **16**, 45–55 (1976)

Turek, F.W., McMillan, J.P., Menaker, M.: Melatonin: effects on the circadian locomotor rhythm of sparrows. Science **194**, 1441–1443 (1976)

Wever, R.: Zum Mechanismus der biologischen 24-Stunden-Periodik. II. Mitteilung. Der Einfluß des Gleichwertes auf die Eigenschaften selbsterregter Schwingungen. Kybernetik **1**, 213–231 (1963)

Wever, R.: Zum Mechanismus der biologischen 24-Stunden-Periodik. III. Mitteilung. Anwendung der Modell-Gleichung. Kybernetik **2**, 127–144 (1964)

Wever, R.: A mathematical model for circadian rhythms. In: Circadian clocks. Aschoff, J. (ed.), pp. 47–63. Amsterdam: North-Holland Publ. Comp. 1965

Wever, R.: Ein mathematisches Modell für die circadiane Periodik. Z. Angew. Math. Mech. **46**, T 148–157 (1966)

Wever, R.: Influence of electric fields on some parameters of circadian rhythms in man. In: Biochronometry. Menaker, M. (ed.), pp. 117–132. Washington D.C.: Natl. Acad. Sci. 1971

Wever, R.A.: The circadian system of man. New York, Heidelberg, Berlin: Springer 1979

Wever, R.A.: On varying sleep-rest schedules: the biological rhythm perspective. In: The 24-hour workday. A symposium on variations in work-sleep schedules. Johnson, L.C., Tepas, D.I., Colquhoun, W.P., Colligan, M.J. (eds.). Washington D.C.: Natl. Inst. Occup. Safety and Health (in press) (1980)

Zimmerman, N.H., Menaker, M.: Neural connections of sparrow pineal: role in circadian control of activity. Science **190**, 477–479 (1975)

Zimmerman, N.H., Menaker, M.: The pineal gland: a pacemaker within the circadian system of the house sparrow. Proc. Natl. Acad. Sci. USA **76**, 999–1003 (1979)