nergic receptors in the intact dog (*8, 12*) and were sufficient to antagonize totally the effects of right accelerator nerve stimulation in the present study. Other less likely receptors that might have played a role in the response, such as alpha-adrenergic and cholinergic receptors, were excluded by utilizing supramaximal doses of the appropriate blocking agents. The most likely explanation for our results, therefore, is that the sympathetic neuroeffector junction involved is fundamentally different from any hitherto described, differing perhaps in the neurotransmitter involved or in the nature of the receptor.

A unique sympathetic neuroeffector junction with uncharacterized pharmacological properties might account for certain inconsistencies in experience with experimental and clinical arrhythmias. In experimentally induced arrhythmias, such as those seen with digitalis intoxication and coronary artery occlusion, considerable evidence indicates that hyperactivity of cardiac sympathetic nerves is a causative mechanism (*13, 14*). However, beta-blocking doses of propranolol have failed to block these arrhythmias (*13, 15, 16*). Clinically, Lown and colleagues (*17*) have described patients whose arrhythmias do not respond to beta-blocking doses of propranolol but do respond to sleep. The explanation for all these findings may be that arrhythmogenic stimuli travel to the heart through nerves whose neuroeffector junctions are not amenable to blockade by conventionally employed antagonists. Stated another way, it seems no longer possible to equate cardiac beta-adrenergic blockade with complete depression of sympathetic nervous activity.

Thus, the demonstration of a unique cardiac neuroeffector junction has important implications for the genesis of arrhythmias and for their pharmacologic therapy. Much may be gained by seeking the transmitter involved in the arrhythmogenic response and then developing antagonists to it. More fundamentally, it raises questions about the adequacy of present knowledge concerning the sympathetic nervous system.

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21 January 1974; revised 6 March 1974

Testosterone Induces "Splitting" of Circadian Locomotor Activity Rhythms in Birds

Abstract. *Under the influence of testosterone, the free-running circadian rhythm of locomotor activity of the starling,* Sturnus vulgaris, *tends to "split" into two components which temporarily run with different circadian frequencies: "splitting" occurred in intact birds whose testes grew, and in castrated birds that were injected with testosterone. Since "splitting" most probably reflects the temporal separation of two (or two groups of) circadian oscillators, these results suggest that testosterone affects the mutual coupling of circadian oscillators controlling locomotor activity.*

It is well established that many biological processes are subject to circadian variations which persist for many cycles under constant environmental conditions. For a long time it has been assumed, explicitly or implicitly, that such circadian rhythmicity reflects the action of *one* "physiological clock" to which the various functions are coupled. Only recently has it become clear that there must be a multiplicity of circadian oscillators within an individual organism, each oscillator controlling a particular set of physiological processes. The strongest evidence in support of this conclusion comes from the observations that (i) a circadian rhythmicity may persist in two or more organs or tissues isolated from one and the same individual (*1–4*) and that (ii) even in an intact organism different circadian rhythms may occasionally run with slightly different frequencies (*1–5*). Therefore, an organism can be considered a population of circadian oscillators, which, while normally synchronized with each other, may uncouple under certain conditions. These findings raise the question how mutual synchrony is normally maintained, that is, which factors are involved in coupling the various circadian oscillators to each other.

Several recent investigations in mammals have made it virtually certain that the daily rhythm of gross locomotor activity, often used as a convenient assay of circadian rhythmicity, is governed by at least two coupled oscillators. This is strongly suggested by the observation that under certain conditions of constant illumination the rhythm of locomotor activity may "split" into two distinct components which run with different frequencies for a while before they resynchronize with each other at a new phase relationship (*3, 6, 7*). This phenomenon has received considerable attention, because it might represent a model case for the study of coupling mechanisms within the circadian system of an organism. However, whereas some external conditions causing "splitting" have been identified, the underlying physiological mechanisms are unknown. The results communicated here suggest that a hormone, testosterone, may promote the "splitting" of circadian locomotor rhythms in the starling, *Sturnus vulgaris*.

The first evidence suggesting that splitting may result from changes in the hormonal state of these birds came from experiments in which groups of 6 to 16 male starlings were transferred

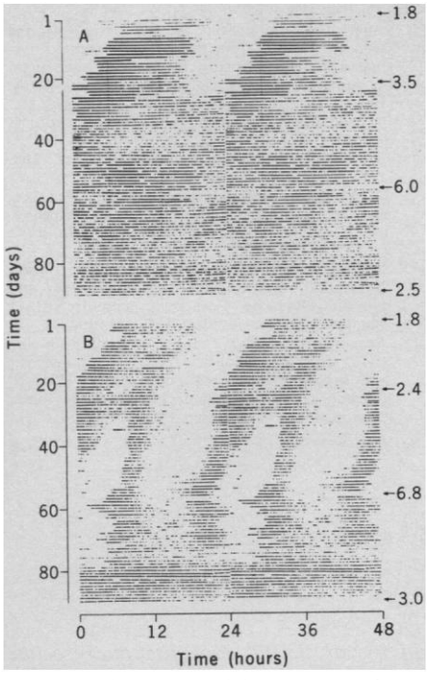

Fig. 1. Circadian activity rhythm of two starlings kept for 90 days under continuous light of about 0.7 lux. Each horizontal line from hour 0 to 24 is the activity record of 1 day. Records from successive days are mounted underneath each other. To facilitate interpretation of the data, the records have been double-plotted on a 48-hour time scale. Each vertical mark indicates the hop of a bird on the perch. During times of intense activity the marks fuse into a solid block. Arrows in the right-hand margin indicate days on which laparotomies were performed; the numbers give the testicular width (in millimeters) measured that day. Activity of the bird shown in (A) is rhythmic during the first part of the experiment, but as testicular size increases the circadian activity time increases also and finally becomes continuous. Activity of the bird shown in (B) is monophasic and rhythmic at the beginning, but with increasing testis size two components become discernible, which subsequently run with slightly different periods, so that they become increasingly separated from each other. Both components increase in duration and finally merge to form one continuous band of activity.

at four times of the year (February, May, August, and November) from outdoor aviaries to constant-condition chambers in which locomotor activity was recorded for at least 3 months (*8*). Testicular size was measured by laparotomy in all birds prior to the transfer to constant conditions and subsequently at about monthly intervals (*8*, *9*). Following the transfer to constant dim light (about 0.7 lux) the testes of all birds in the experiments starting in November and February increased in size, whereas the testes of all 31 birds of the experiments starting in May and August either decreased (May) or remained in their small regressed state (August) (*10*). In these experiments a strong positive correlation was found between testicular size and daily activity time (α): α increased in the birds of the November and February groups whose testes grew, it remained small in the birds of the August group whose testes remained inactive, and it decreased in the birds of the May group whose testes regressed (*8*). In many of the birds of the November and February groups the steady increase of α resulted finally in continuous activity, and circadian rhythmicity was no longer apparent (Fig. 1A). In three of these birds the state of continuous activity was preceded by a state of "splitting." In these birds the previously unimodal activity pattern became gradually separated into two components which for some time showed distinctly different circadian frequencies. Both components gradually increased in duration until they finally merged into one continuous band of activity (Fig. 1B).

These results suggested that the development of continuous activity and the preceding state of "splitting" observed in birds with growing gonads may have been caused by changes in the birds' hormonal state related to testicular recrudescence. To test this hypothesis, two basically identical experiments were started in December 1971 and February 1973. A total of 28 male starlings were castrated (*9*) and subsequently kept for 69 to 80 days in constant condition chambers in which light intensity was kept constant at about 0.2 lux (*8*). Under these conditions α remained essentially constant and no signs of "splitting" were observed in any of these birds. Between days 70 and 81, 15 of these birds were injected with 2.5 mg of testosterone dissolved in 0.1 ml of sesame oil, usually at about weekly intervals, whereas the remaining birds were injected with 0.1 ml of sesame oil only (*9*). No changes in activity time were observed in the birds injected with sesame oil (Fig. 2A). In contrast, all birds injected with testosterone increased α (Fig. 2, B and C) and eight individuals showed, at least temporarily, signs of "splitting" (Fig. 2, D to F). In three of the eight birds in which "splitting" was observed activity finally became continuous. In three birds the activity remained in a "split" state until the experiment was terminated (Fig. 2D).

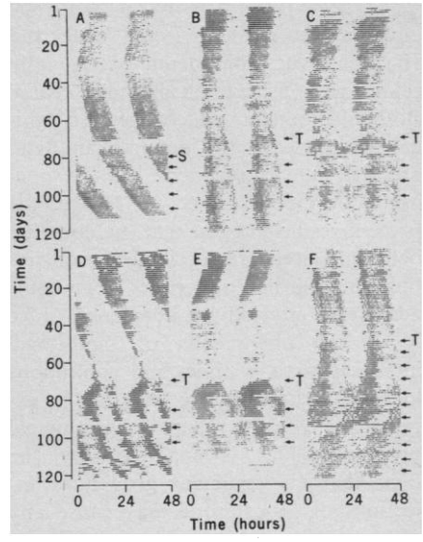

Fig. 2. The effect of testosterone on the locomotor activity rhythm of male starlings kept in conditions of continuous illumination. All birds were castrated prior to the beginning of the experiment and injected with sesame oil (*S*) or testosterone (*T*) on the days indicated by arrows at the right-hand margin. (A) Record of a control bird injected with sesame oil. (B–F) Records of birds injected with testosterone. Following the first testosterone injection, activity lengthens in birds (B) and (C) and "splits" into two components in birds (D), (E), and (F). In (E) and (F) the second component finally merges with the first component in such a way that its beginning temporarily represents the onset of the single activity time. Injections of sesame oil have no effect on the activity rhythm of bird (A). For further explanations see Fig. 1.

And in two birds the two components eventually rejoined after they had scanned an entire cycle (Fig. 2, E and F). No clear effects of the various treatments on the net circadian period length of the system were apparent.

Several tentative conclusions may be drawn from these results:

1) "Splitting" of the circadian activity rhythm of the starling can be provoked by testosterone. By this statement nothing is said about the mode of action of testosterone, that is, whether testosterone acts directly on the circadian system or indirectly by interfering with other components of the neuroendocrinological system. Nevertheless, the results support the general hypothesis that "splitting" may be under hormonal control (*7*).

2) "Splitting" most probably reflects the temporal separation of two (or two groups of) circadian oscillators. This is strongly suggested by the facts that (i) the two components show different

circadian periods for some time and (ii) the two components occasionally rejoin with a new phase relationship. Both phenomena, which are also typical for some of the mammalian data, are difficult to reconcile with a one-oscillator model (*3, 6, 7*). Hence, the present results suggest that the coupling between circadian oscillators controlling the diurnal pattern of locomotor activity is effected by hormones.

3) The phenomena of "splitting," increased activity time, and continuous locomotor activity are apparently expressions of the same physiological processes. This is suggested by the facts that (i) testicular growth or injections of testosterone may cause any of these three phenomena in different individuals and (ii) increasing activity time or developing continuous activity are often the result of or preceded by "splitting." Therefore, the present data suggest that the development of continuous activity is at least partly a consequence of the dissociation of two or more circadian oscillators.

Continuous and apparent arhythmic activity patterns have also been observed in various animal species kept under high intensities of continuous illumination and in birds in which the pineal gland had been surgically removed (*11, 12*). It has been suggested by several authors that continuous activity under these conditions results from the uncoupling of two or more circadian oscillators controlling locomotor activity (*3, 7, 11*). The present data support this hypothesis.

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9. Testicular size was established by making a small incision between the last two ribs on the left side of the bird, locating the testis, and measuring its width with a compass or calipers. Castration was accomplished by making the same incision on both sides of the bird and removing the testes with a pair of forceps. In a few cases regenerating testicular tissue was found when the birds were laparotomized on termination of the experiments. Data from such birds are not included in this report. Testosteronoenanthat (Schering AG, Berlin) was used. Injections were given into the pectoral muscle. The effectiveness of the testosterone injections was witnessed by the fact that the previously black bill coloration turned yellow starting about 4 weeks after the first injection. Yellow bill coloration has previously been shown to be under the control of testosterone [H. E. Witschi and R. A. Miller, *J. Exp. Zool.* **79**, 475 (1938)].
10. Such a differential testicular response to continuous light was expected on the basis of other photoperiodic studies on starlings and other Temperate Zone birds. It reflects the seasonal change from photoresponsiveness during winter and spring to photorefractoriness in summer and fall [D. S. Farner and R. A. Lewis, in *Photophysiology*, A. C. Giese, Ed. (Academic Press, New York, 1971), vol. 6, p. 325].
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Localization of Sister Chromatid Exchanges in Human Chromosomes

Abstract. *The bromodeoxyuridine sensitivity of 33258 Hoechst fluorescence allows microfluorometric analysis of sister chromatid exchanges in human metaphase chromosomes. The frequency of sister chromatid exchanges among chromosomes correlates with chromosome length. Exchanges appear to occur predominantly in interband regions, as defined by quinacrine fluorescence, or very near band-interband junctions. A few regions are involved unusually frequently.*

Metaphase chromosomes of a number of organisms exhibit distinctive quinacrine and modified Giemsa (*1*) banding patterns. The bands, which exhibit bright fluorescence with quinacrine and stain intensely with Giemsa, appear to consist predominantly of heterochromatin (*1, 2*). Heterochromatic regions have long been considered sensitive to damage by a variety of agents (*3*), although recent studies suggest that high energy radiation breaks human chromosomes principally in the interband regions (*4*). Chromosome breaks, however, may represent only that small fraction of damaged regions that have not undergone repair.

Sister chromatid exchanges are events that involve breaks in both chromatids at coincident locations with subsequent

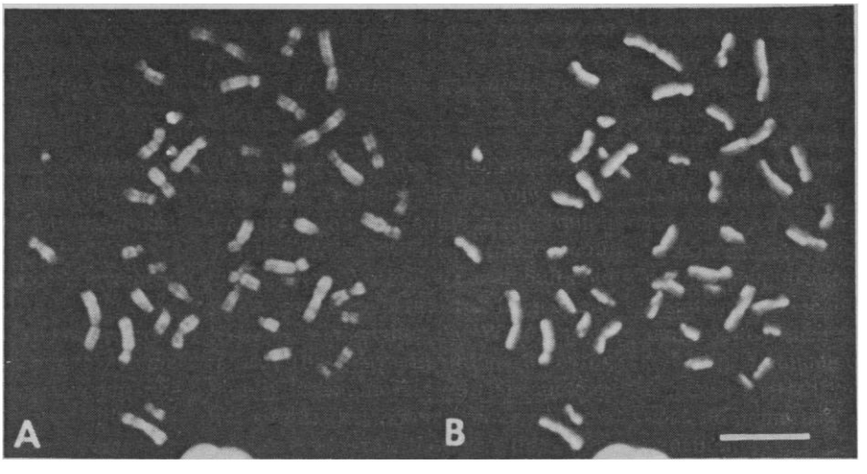

Fig. 1. Quinacrine and 33258 Hoechst fluorescence of human chromosomes from cells grown two divisions in medium containing 5-bromodeoxyuridine (BrdU). Chromosomes were prepared from peripheral leukocytes of a normal human male (46,XY) which were grown 72 hours in medium containing 0.02 m*M* BrdU. (A) A metaphase was stained first with quinacrine and photographed, and (B) destained, then stained with 33258 Hoechst, and photographed. The former (A) displays a characteristic quinacrine banded fluorescence pattern while the latter (B) exhibits the 33258 Hoechst fluorescence pattern expected after two cell divisions in medium containing BrdU. The bar in (B) represents 10 μm.