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The Stability of the Circadian Rhythm of Green Finches (*Carduelis chloris*) under the Influence of a Weak Electrical Field[1]

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Abstract Free-running activity rhythms of nine green finches (*Carduelis chloris*) were studied under the influence of a 10-Hz square-wave electrical field. With a field strength of $|E| = 2.5$ V/m in the empty cage, the population had a mean period of 23.64 ± 0.77 hr. In the same experiment, but without the electrical field, the period was 23.66 ± 0.80 hr. These results are in contradiction to Wever's description of a field-induced shortening of the period. A series of experiments with 10-Hz pulses of the same square-wave form, yet with various field strengths (8.7 and 65.2 V/m), also gave no effects.

Changes in free-running rhythms, under the influence of a weak nonionizing electrical field with square waves of 10 Hz at a field strength of $|E| = 2.5$ V/m, have been reported for humans (Wever, 1968a, b) and also for green finches (*Carduelis chloris*) (Wever, 1973, 1985). The circadian system of both species showed a shortening of its spontaneous period, τ, under the electrical field. In addition to this, the field reduced the number of states of internal desynchronization in humans and acted as a zeitgeber. The field led to a highly significant shortening (average 1.3 hr) of the period in human subjects; in *C. chloris,* the corresponding value was 0.8 hr. These results led Wever (1973, p. 231; 1985, p. 512) to the general conclusion that the field effect was not restricted to humans. With the exception of experiments that showed phase delays in the white-footed mouse (*Peromyscus leucopus*) under an alternating-current 60-Hz field of 100 kV/m (Ehret and Duffy, 1983), no other results involving electrochronobiological interactions have been reported. Further detailed studies of the effects of low-frequency fields, with different waveforms and amplitudes, on τ were therefore necessary to augment these results. To test the model and the experimental set-up, we started the examination with the 10-Hz square-wave field used earlier by Wever.

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MATERIALS AND METHODS

BIRDS, HOUSING, AND ELECTRICAL AND RECORDING EQUIPMENT

The main experiment was performed with the 10-Hz square-wave field at a field strength of $|E| = 2.5$ V/m. The field strengths are reported in this paper as "peak-to-peak" values. The nine green finches (*C. chloris chloris L.*) (four females, five males) used in this experiment were obtained from a breeding colony (Schmidt, 6601 Riegelsberg, Federal Republic of Germany). The birds were all 1–2 years old and were made familiar with the conditions of the experiment for more than 6 months. At the end of the studies, they were returned to the breeder in good condition. The birds were housed individually in wooden cages (covered with organic webbing) identical to those used by Wever. The light intensity was kept constant (LL) and measured at the height of the perches (see Table 1, below). The finches were provided with wild seed continuously and with tapwater every 3 days at different times of the day. Each cage was placed in a sound-damping wooden box, which was opaque and ventilated. To improve each bird's temporal isolation and to avoid social communication, an acoustic white-noise generator was used.

Two grid electrodes ($\geqslant$9 cm wider than the base of the cage) were placed outside each box. The lower grid was grounded at all times, but the upper grid was grounded only during zero-field conditions. Shielded cables led from each pair of grids to a function generator (rise time $\leqslant 5$ μsec; pulse duty factor 0.5). The stability of the signal frequency was continually controlled by a counter. The waveform amplitudes were occasionally monitored by an oscilloscope and adjusted accordingly. The vertical component of the field was measured in each empty box by a calibrated electrometer probe (Bach and Lang, 1976; Pfützner, 1979). The required electrical field, which had a symmetrical bipolar pulse form, was found by probe measurements in the empty cage. The direct-current field was set at 0 V/m. The experiments took place in an underground laboratory—the "Bunker" of the Max-Planck-Institute of Psychiatry in 8138 Andechs, Federal Republic of Germany, which is electromagnetically shielded by welded steel reinforcement and five jointless layers of hot-rolled transformer sheet iron. In order to minimize biological effects, all electrical devices and recording equipment were located outside the laboratory. Nevertheless, a low-frequency electrical field with a strength of about 1 V/m could be measured in the room; all efforts to reduce this field failed.

The locomotor activity was recorded over 2-min periods, using microswitches under the perches connected to a personal computer. The recording on the left side of Figure 1 (below) shows the raw data, in which black marks represent an active bird. The experiment was performed from October 1987 to February 1988. The two 10-Hz-field sections and the three control sections all lasted at least 21 days. The field was twice switched on simultaneously for all birds.

Preliminary experiments were performed with green finches kept in polyethylene cages. In these experiments, ultrasound sensory systems registered the birds' locomotor activity. The 10-Hz square-wave field was applied with field strengths of 8.7 and 65.2 V/m.

EVALUATION OF THE DATA

In order to accurately define the onset of each activity cycle, the first 2-min time interval that followed 1 hr of average activity above a particular threshold was taken as significant. The right side of Figure 1 shows the activity recording transformed by this procedure. The cycle duration is the difference between two successive onsets of activity. For each finch, τ_0, the mean period for all the cycles without field, was determined. The mean period with the field in operation, τ_{10}, was calculated analogously. The difference between both values for each bird was found by this formula: $\Delta\tau = \tau_0 - \tau_{10}$. For the whole population, the mean periods $\bar{\tau}_0$ and $\bar{\tau}_{10}$ (with standard deviations s_0 and s_{10}, respectively) were calculated for both conditions. $\Delta\,\bar{\tau} = \bar{\tau}_0 - \bar{\tau}_{10}$ was the mean difference between all measured periods for the whole population under the influence of all zero-field and 10-Hz-field sequences; s_Δ is the related standard deviation.

As each bird was observed over continuous time intervals under changing field conditions, the samples are connected. The Student's t test for paired differences was applied to the nine differences, $\Delta\tau$, at the significance level of $\alpha = 0.05$. The one-tailed test with the alternative hypothesis (H_1), $\bar{\tau}_0 > \bar{\tau}_{10}$, was used.

RESULTS

In the main experiment, the nine green finches showed no overall field-dependent changes in their free-running circadian rhythms (Table 1). The stability of their rhythms is clearly demonstrated by birds 2 and 9 (Fig. 1); each bird yielded nearly identical τ_0 and τ_{10} values. Other effects, such as phase shifts or changes in the amount of activity, could not be found in the recordings. The average intensity of light, $\bar{y}$ (in lux), was 0.9 lux (Table 1). The population showed a mean period of $\bar{\tau}_0 =$ 23.66 hr, with $s_0 = 0.80$ hr, under zero-field conditions. Under 10-Hz-field conditions, the mean period was $\bar{\tau}_{10} = 23.64$ hr, with $s_{10} = 0.77$ hr. The mean difference, $\Delta\,\bar{\tau}$, calculated from $n = 9$ paired differences, was 0.02 hr. As the test statistic $\hat{t} = 0.34 \leq 1.86 = t_{\alpha,\nu}$ (ν = degrees of freedom = $n - 1 = 8$), the alternative hypothesis was rejected at the level of significance α.

During the preliminary experiments, only small $\Delta\tau$ values, inconsistent in sign, were detected when the 10-Hz-field conditions were changed. For example, a population consisting of seven finches showed a mean increase of $\Delta\,\bar{\tau} = 0.06$ hr when the field ($|E| = 8.7$ V/m) was switched on; a similar result was found with the other field strength used.

DISCUSSION

The experimental conditions in our preliminary experiments deviated in three respects from those used by Wever. These deviations concerned the applied field strengths of the 10-Hz field, the technique of recording the birds' activity, and the

TABLE 1. Periods for Each of Nine Green Finches and for the Whole Population, without (τ_0, $\bar{\tau}_0$) and with (τ_{10}, $\bar{\tau}_{10}$) an Electrical 10-Hz Square-Wave Field of $|E| = 2.5$ V/m

Bird no.	Light intensity (lux)	τ_0 (hr)	τ_{10} (hr)	$\Delta\tau = \tau_0 - \tau_{10}$ (hr)
1	1.3	23.89	23.66	0.23
2	0.6	24.53	24.54	−0.01
3	0.6	22.75	22.72	0.03
4	1.8	24.58	24.81	−0.23
5	0.4	24.10	23.79	0.31
6	0.5	22.21	22.43	−0.22
7	0.8	24.00	23.83	0.17
8	0.9	23.18	23.27	−0.09
9	1.2	23.74	23.74	0.00
$n = 9$	$\bar{y} = 0.9$	$\bar{\tau}_0 = 23.66$ $s_0 = 0.80$	$\bar{\tau}_{10} = 23.64$ $s_{10} = 0.77$	$\Delta\bar{\tau} = 0.02$ $s_\Delta = 0.19$

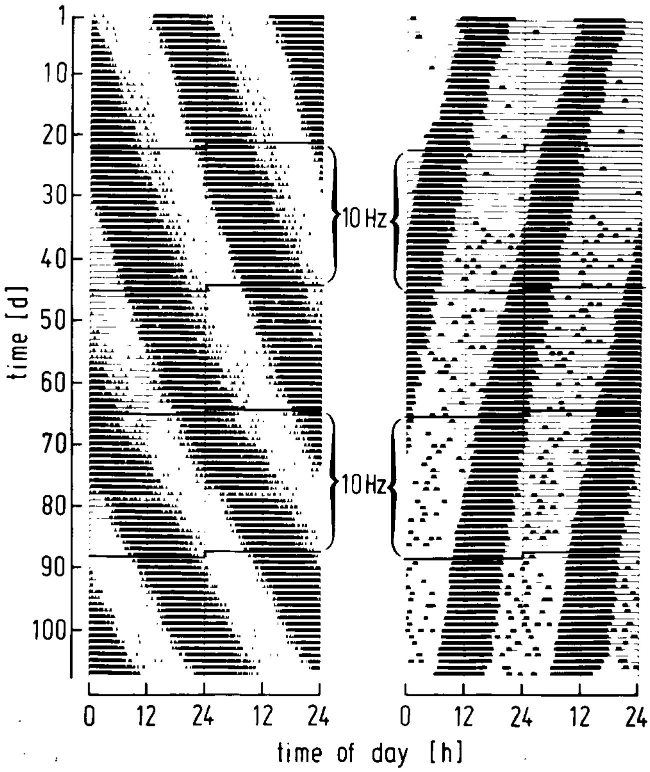

FIGURE 1. Free-running activity rhythms of two green finches (*Carduelis chloris*) under LL conditions, kept alternately with and without an electrical field (10 Hz, square-wave; 2.5 V/m). Marked areas indicate times when the field was in operation. Left: Original record for bird 2. Right: Transformed data for bird 9. Both records are duplicated.

material of the cages. Because unequivocal field-induced changes in τ were not found, it seemed possible that the missing effect could be due to the above-mentioned deviations. Therefore, for the crucial main experiment, we reproduced all of Wever's original experimental conditions in the same laboratory; the intensity of light in LL was set, after personal communication with the author, at approximately 1 lux. Under zero-field and 10-Hz-field conditions, the population showed nearly identical mean periods ($\bar{\tau}_0$ and $\bar{\tau}_{10}$, respectively). The nine paired differences did not differ significantly in the t test ($p = 0.35$, where p gives the probability that the value of $\hat{t}$ is caused by random variation). There were no other indications from our data that could have supported the hypothesis that the applied 10-Hz field would cause any other distinct effects. The field was only applied in three low-level field strengths, as this range seemed to be of primary interest. However, this could be criticized in the light of the present results.

To explain the dissimilarity between the results reported here and in the earlier investigation, the principal question to be raised is whether the changes in τ Wever observed have to be interpreted exclusively with regard to the field. The spontaneous variation in τ was examined for the house sparrow (*Passer domesticus*) (Eskin, 1971). For the first 60 days in constant darkness, the sparrows showed a remarkably high intraindividual variability in τ. Therefore, it seems possible that the change Wever observed was endogeneous and not necessarily a result of the field. Such intrinsic changes could be traced back to aftereffects of previous photoperiod and/or neuroendocrine adaptations related to season. Furthermore, these changes were even smaller than the endogenous drifts in τ that Eskin published. This interpretation could explain, within the small number of Wever's (1973, 1985) experiments, the varying results of both field studies. However, under this assumption, any empirical basis for accepting the hypothesis of electrochronobiological interaction caused by the 10-Hz field is missing in green finches.

Besides this, one has to ask whether the circadian system is sensitive to weak physical factors. In homeothermic species, the remarkably low dependence of τ on the ambient temperature is well known. The homeostasis of circadian oscillations is likewise reflected in their resistance to most chemical manipulations. Only deuterium oxide, several sex hormones, and certain neuropharmacological substances have been shown to affect the frequency of circadian rhythms (Wirz-Justice *et al.*, 1982). If the free-running period is so stable, one has to ask how a weak electrical field could have the hypothesized effects. The present results provide further experimental confirmation of the resistance of circadian timekeeping to external stimuli such as weak electrical fields.

On the other hand, Wever's field experiments on humans suggest an affirmative answer to this second basic question. However, there is no plausible "mechanism of action" for the effect of such weak electrical fields. Schaefer (1983, p. 86) points out that humans have no known sensory organ "four times more sensitive than the most sensitive Lorenzini ampule" (our translation). Although electroreceptors similiar to those in the lateral line system of fish have not been found in humans, an alternative mechanism of sensory perception may exist. However, Wever's results with humans have not as yet been replicated, in spite of the significance they may have.

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