Further Evidence for Period Lengthening Effect of Li^+ on Circadian Rhythms

Karlfriedrich Hofmann, Martina Günderoth-Palmowski, Gottfried Wiedenmann, and Wolfgang Engelmann

Institut für Biologie I, Tübingen

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The period length of the activity rhythm of the cockroach *Leucophaea maderae* is increased by a LiCl solution of 100 mol m^{-3} offered as drinking water by about 1%.

Li^+ increases the period length of circadian rhythms in the petal movement of *Kalanchoe blossfeldiana* [1]. The critical daylength in photoperiodic flower induction of *Pharbitis nil* and *Chenopodium rubrum* is also increased by Li^+ [2]. Since, according to Bünning [3], a circadian oscillator is responsible for the measurement of daylength in photoperiodic reactions, this finding was used as a further indication of Li^+ affecting circadian rhythms. Li^+ is known to influence the permeability of membranes [4] and the activity of membrane bound enzymes [5]. The period lengthening effect of Li^+ has been used therefore as evidence for the involvement of membranes in the mechanism of circadian rhythms [6].

The effect of Li^+ on circadian systems in animals was, however, not studied except for some preliminary results [1] and speculations of its effect on circadian rhythms in humans particularly in depressive disorders [7].

Because of this lack of information we have undertaken a more detailed study of Li^+ affecting the activity rhythm of cockroaches and – in a preliminary way, of hamsters (*Mesocricetus auratus*). Some of the results are reported here.

Methods

Leucophaea maderae, originally from Lohmann [8], was reared in our laboratory since 1969. The animals were kept in LD cycles 12:12 at 22.5 °C. Only males were used for the experiments. The activity rhythm was measured in running wheels in which the animals were housed individually with access to food and water. The running activity was recorded with an event recorder under red light and a temperature of 28 °C ± 1 °C. Actograms were prepared and the period length determinated in the usual way [9]. LiCl solution was offered at irregular times for about 1 hour daily. During the time of Li^+ treatment no normal water was available. No synchronizing or period changing effect was found in the controls which were treated in the same way except without LiCl in the water. Animals which died during the experiment were air dried and used to determine the concentration of Li^+ in the body. A Beckman 1272 digital atom absorption spectral photometer was used.

Requests for reprints should be sent to Dr. Wolfgang Engelmann, Institut für Biologie I, Auf der Morgenstelle 1, D-7400 Tübingen.

Results

We have offered 10, 50, and 100 mol m^{-3} of an aequous LiCl solution in two experiments to altogether 31 animals. Fig. 1 shows actograms for 4 examples. In Fig. 1 a an increase in period length is visible immediately after onset of the Li^+ treatment (arrow). This is found in about 22 out of 31 cases and more marked at higher doses. Fig. 1 b shows an actogram of one of 3 cases in which the period was further increased about 3 weaks after onset of Li^+ administration. Fig. 1 c shows another rare kind of response, in which the period lengthening occured about 3 weeks after onset of the Li^+ treatment. This was found in 4 cases. In 5 cases no period lengthening effect of Li^+ on the free runnning rhythm was found. Such an example is given in Fig. 1 d. In 2 of the 5 cases this was also found in animals receiving more than 10 mol m^{-3} LiCl. In one case (not illustrated) an extraordinary strong response to Li^+ was found, with a period lengthening of 0.7 hours. The weight of this animal was exceptionally low (0.28 versus 0.55 g ± 0.03 g standard error as the average dry weight of the rest of the animals).

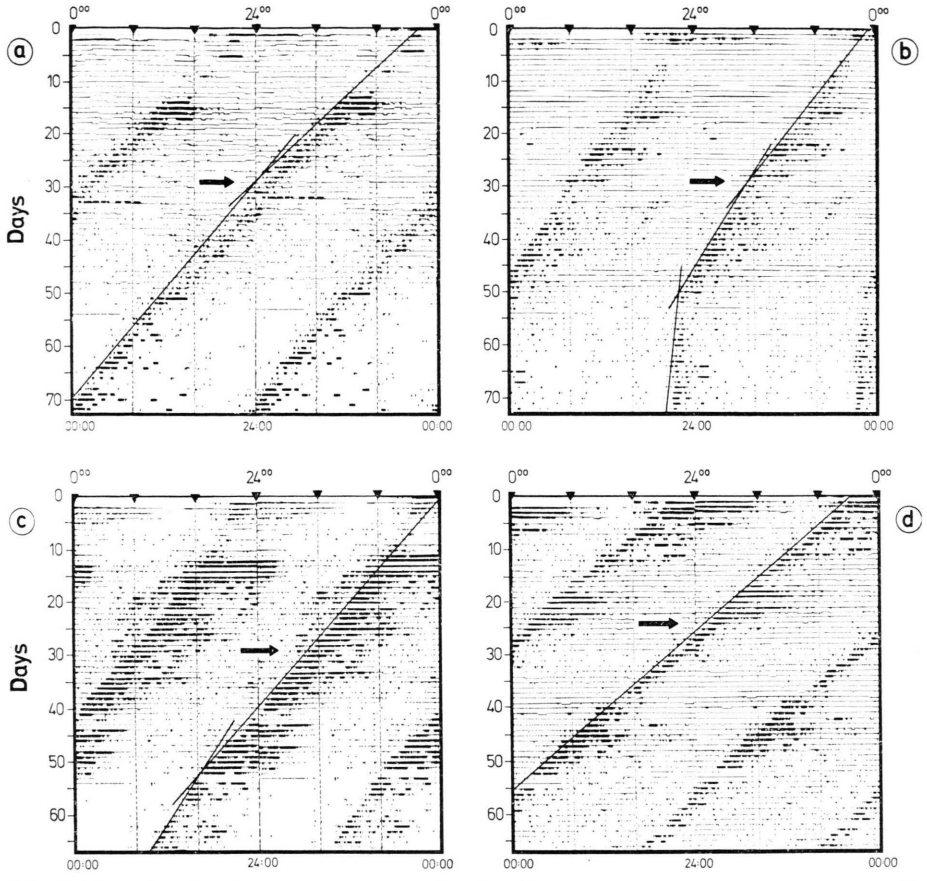

Fig. 1. Examples for the effect of LiCl on the circadian activity rhythm of *Leucophaea maderae* under constant conditions of temperature and light (28 °C, weak red light).

a: immediate period lengthening by 0.2 hours after offering 10 mol m^{-3} LiCl in the drinking water, from tip of arrow onward;

b: immediate period lengthening by 0.2 hours after offering 10 mol m^{-3} LiCl in the drinking water, and further lengthening by 0.5 hours after about 3 weeks;

c: period lengthening effect of 0.15 hours occurs about 26 days after onset of Li^+ administration (50 mol m^{-3});

d: no period change after 10 mol m^{-3} LiCl was offered to the animal.

All actograms shown are doublicated to facilitate the determination of the period length. Onsets of activity are connected with eye fitted lines.

The period lengthening effect is shown in Table I for the different Li^+ concentrations as mean increase in period. A one tailed WILCOXON matched pairs signed-ranks test [10] shows significance at the 0.025 level for the 10 mol m^{-3} group, and at the 0.005 level for the 50 and 100 mol m^{-3} groups.

In most cases the period decreases again after the end of the Li^+ treatment, although in 6 out of 14 cases the period length was not decreased to the original value before the Li^+ treatment. However, only in the group with the lowest concentration enough animals were still alive for a significance test, and the α value found was between 0.01 and 0.025.

Since some of the animals treated with Li^+ did not show any period lengthening and since this was found not only in the group receiving low concentrations of LiCl, we determined the Li^+ concentration in the animals after they had died. Only 14 out of 18 (second experiment only) were analysed. The rest was still alive. With the number of animals checked, we did not find a significant correlation of the period length with the internal Li^+ concentration (SPEARMAN rank correlation coefficient $r_S =$

Table I. Mean increase ($+\Delta\tau$) of period length of activity rhythm in *Leucophaea maderae* (2nd column) as a function of the LiCl concentration [Li^+] in the drinking water (1st column). Mean decrease of period ($-\Delta\tau$) after LiCl solution was replaced by water again (5th column). 4th column: number of non responders.

[Li^+] [mol m^{-3}]	$+\Delta\tau$ [h]	N	N of no $\Delta\tau$	$-\Delta\tau$ [h] after Li treatment	N
10	+0.11	12	3	−0.06	10
50	+0.09 *	10	1	−0.1	2
100	+0.18 *	9	1	−0.1	1

* Significant at the 0.5% level.

0.425. For $\alpha = 0.05$ a r_S of 0.600 would have been necessary [10]) even after correction for different weight and different length of application of the Li^+.

Death rate was higher and life expectancy shorter for the animals treated with higher concentrations of Li^+: 30 days after onset of Li^+ treatment about 30% of the animals in the 10 mol m^{-3} group died, whereas in the 50 mol m^{-3} group 50% and in the 100 mol m^{-3} group about 85% died.

Discussion

The results show a small but significant period lengthening effect of LiCl on the activity rhythm of cockroaches at concentrations of 50 and 100 mol m^{-3} in the drinking water. We did not find a significant correlation between the concentration of Li^+ in the animals and the period lengthening. However, the number of animals analysed was rather low. In some animals no change in period was found. This could have been the result of a low internal Li^+ concentration, perhaps due to the ability of cockroaches to use metabolic water as known from other insects [11]. The determination of the internal Li^+ content showed, however, that some animals (2 out of 10) with no change in period did possess Li^+ contents comparable to those which lengthened the period. We do not know, whether in these cases Li^+ did not reach the place of action on the circadian rhythm, whether genetic differences are involved, or whether other explanations have to be found. This holds also for the few cases in which a second further increase in period was found several weeks after the initial Li^+ effect. In the only case in which a very considerable and immediate period change of 0.7 h was found, the weight of the animal was exceptionally low.

The period lengthening effect of Li^+ seems to be reversible after the Li^+ treatment was stopped. However, in 3 out of 7 cases the period length did not decrease immediately after water had replaced the Li^+ solution. Since most of the animals died during the Li^+ treatment, we do not have sufficient data for a more detailed analysis.

The period lengthening effect of Li^+ on circadian rhythms seems thus to be a phenomenon not only restricted to plants. The data presented here add an insect to the list of organisms responding to Li^+ with the period lengthening of one of its circadian rhythms. Earlier a case of lengthening the period of circadian activity rhythm in a rodent (*Meriones*) was described [1]. This was, however, based on a few cases only. We have

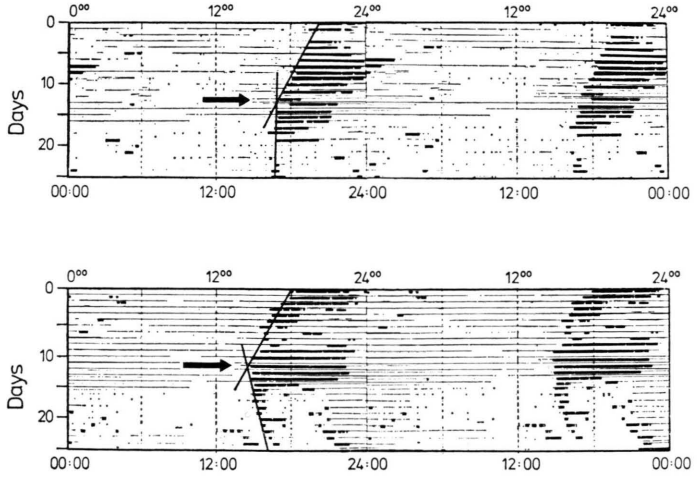

Fig. 2. Actograms of male hamsters (*Mesocricetus auratus*) 6 weeks of age at the begin of recording in running wheels. Constant conditions of temperature (22.5 °C) and light (weak red). LiCl was added to the drinking water from the 13th day onward in concentrations adjusted to the body weight (about 6—9 mg per day).

started to repeat the experiments with rodents using *Mesocricetus auratus* in running wheels and found similar results which are shown in Fig. 2. Out of 4 animals tested one showed a rather irregular activity rhythm which did not allow to measure the period length. Another animal which had a period length greater 24 hours before the Li^+ treatment started, did not respond to Li^+. The other 2 animals, both free running with less than 24 hours before adding Li^+ increased the period by about 1.5% after Li^+ administration. Some details of the experiment are described in the legend to the figure. However, the experiment has to be taken as a preliminary one, and we are currently testing a larger number of hamsters for its response to Li^+.

These experiments are important in respect to recent speculations and some experimental findings [12] according to which in endogenous depression of humans the circadian system might function unproperly. If, as suggested by Pflug *et al.* [12], a shorter period of a circadian rhythm appears under the normal 24 hour day during depression, the period lengthening effect of Li^+ might bring this rhythm in the range of entrainment. However, many other explanations are feasible, and experimental results are urgently needed on the effect of Li^+ on the circadian system of humans in order to reduce the great number of hypothesis.

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