Effect of Lithium Chloride on the Circadian Rhythm in the Flight Activity of the Microchiropteran Bat, *Taphozous melanopogon*

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We have performed experiments on the influence of Li^+ offered through drinking water i) on the period length (τ) of the rhythm in the flight activity of bats under LL and ii) on phase angles during entrainment under LD cycles. Bats subject to entrainment exhibited *no* qualitative and quantitative change in ψ values after Li^+ treatment. In contrast the ingestion of Li ions by bats under free-running conditions shortens the τ of the bat rhythm by an amount that is a function of period length prior to ingestion. τ is, thus homeostatically conserved in the face of Li administration in bats.

Circadian rhythms persist in the absence of environmental cycles but may be entrained with an oscillatory parameter such as a light and temperature cycle [1]. The period of the circadian oscillations remains remarkably stable under these conditions, except in the face of a very restricted number of chemical and pharmacological challenges. Active chemical substances that interfere with the rhythmic mechanisms are of the nature of ions, ionophores, uncouplers and anesthetics [2, 3]. It has been hypothesized that ionic balance across cellular membranes are influenced by these chemicals and are thus parts of the clock mechanism. Recent findings of ions being involved in circadian rhythms support this view [3, 4].

Lithium ions were first reported to cause period lengthening, in the circadian rhythms of petal movement of a crassulacean flower and in the locomotor activity of a desert mouse [5] and has since been shown to have significant effects in several other systems [6]. In all these cases a period lengthening effect has been reported. I have studied the influence of Li^+ on the flight activity rhythm of the bat, *Taphozous melanopogon* offered through drinking water. The period of the circadian rhythm of this bat somewhat unexpectedly shortened and the oscillation is apparently speeded up. Li^+, however, does *not* cause any change of the phase angle of the entrained rhythm under artificial light-dark cycles (LD 12:12 h).

Methods

Bats were housed in spring-suspended cages (30 × 30 × 30 cm). Locomotor activity of these bats was monitored with microswitches which activated writing pens of a 20-channel Esterline Angus Event Recorder. The experimental animals were held under constant fluorescent light (LL) and temperature (30 °C ± 1 °C). Light intensity, however, varied between 2.5 lx and 6.5 lx depending on the position of the cage. Desired regimes of light and darkness were obtained with the aid of a time switch. The entrained and free-running periods of activity were measured by drawing a line that best fitted the slope of the activity bands at its onset.

Stable values of period length (τ) were established while bats were in LL and drinking tap water. After 16 days of free-run, the drinking dishes were then replaced with 0.75% LiCl solution. Li^+ administration (3.7 ml/bat/day; mean value) continued for the consecutive days of the experiment. (In hamsters and squirrels the amount of Li^+ solution consumed, however, decreases over the days; Prof. W. Engelmann, personal communication.) Final steady values of τ obtained while bats were ingesting Li^+ were calculated and compared to the τ prior to Li^+ administration.

Abbreviations: LL, continuous light; LD, light-dark cycles; DD, continuous darkness.

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Results

Effects of Li⁺ in LD 12:12 h

Animals exposed to LD cycle (12:12 h) exhibited a single burst of activity for 9 to 10 h during the dark periods. The onset of activity relative to "light-off" was delayed and ψ (phase angle of onset of activity) values during entrainment were negative (Fig. 1). The animals treated with Li^+ (0.5%; 0.75%; 1% concentrations) – like the controls-entrained to LD 12:12 h with a phase angle difference. However, striking differences in the change of ψ were not apparent (Fig. 1). The mean ψ (−17.3 min ± 7.2 S.E.) values under LD before Li^+ treatment did not differ significantly relative to ψ values (−19.6 min ± 12.2 S.E.) while they were ingesting Li^+.

Effects of Li⁺ in LL

The activity recording of Li^+ treated animal is shwon in Fig. 2. It can be seen that Li^+ (0.75%) had major effects on the free-running circadian activity rhythm. The comparison of values from 16 day intervals before (τ_1) and after (τ_2) the Li^+ ingestion reveals that τ_2 was shorter than τ_1 (Fig. 2). This

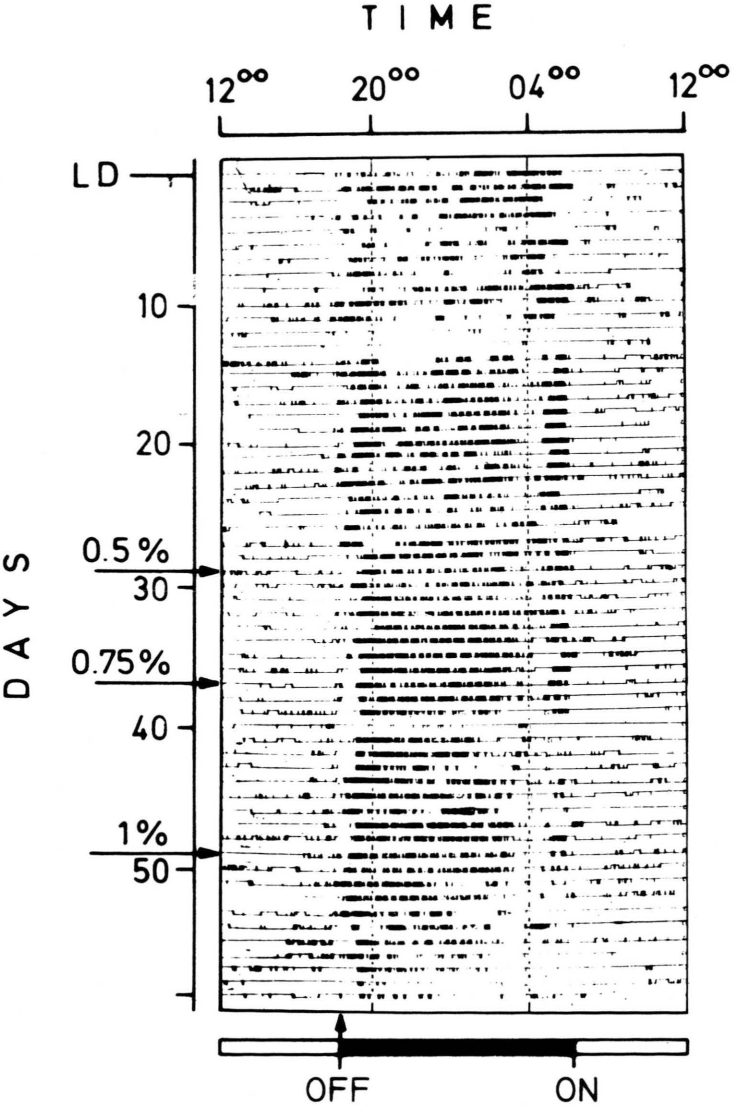

Fig. 1. Raw data showing the effect of 0.5%, 0.75% and 1% lithium chloride on the phase angle difference of locomotor activity in a bat under LD (12:12 h). The time scale runs from left to right with successive days' activity shown beneath each other. The abscissa is time of day in hours and the ordinate is time in days. The animals treated with Li^+ did not show any change of phase angle difference. The slight advance of phase angle difference of entrained activity rhythm from days 44 to 60 is spontaneous which almost equals to the control animal.

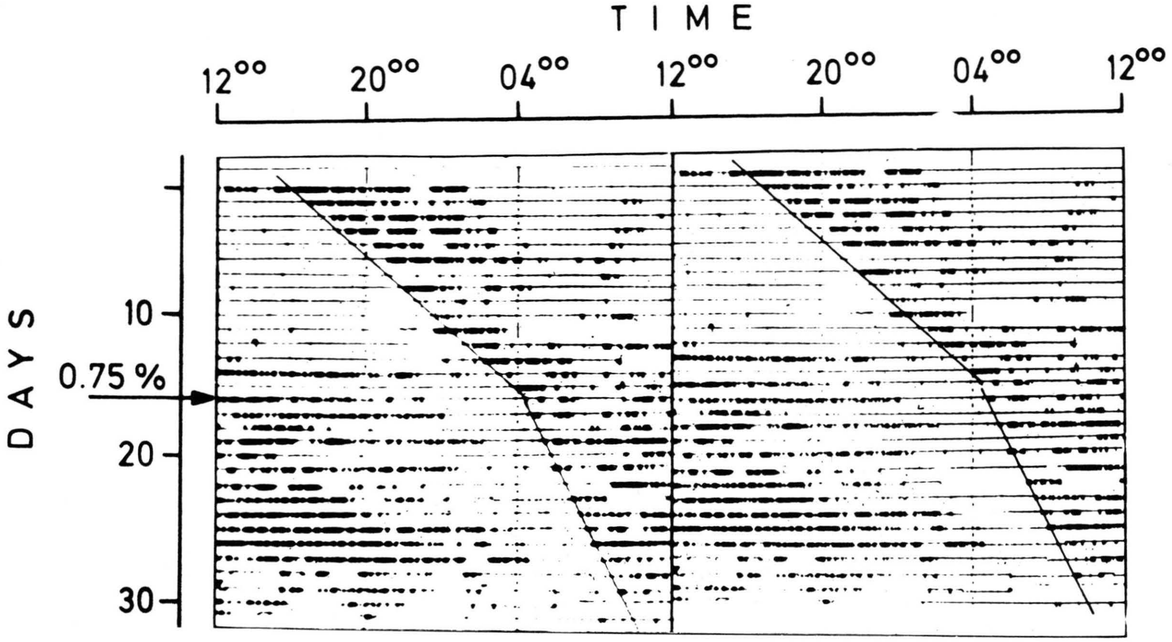

Fig. 2. Effect of Li$^+$ on the circadian activity rhythm of *Taphozous melanopogon* in continuous light (LL) and temperature. Period shortening occurred immediately after offering 0.75% of LiCl in the drinking water on day 16. The arrow indicates the day on which LiCl was offered. The aktogram shown is double plotted to facilitate the determination of the period length. Onsets of activity are connected with eye fitted lines.

decrease in the period length is visible immediately after the onset of Li$^+$ treatment.

The shortening of τ in response to Li$^+$ administration, however, involves greater complexity than a mere change from τ_1 to τ_2. There was a great range of inter-individual variation in the periods expressed before Li$^+$ administration. Similarly the range of values of τ_2 and hence the magnitude of variation $\Delta\tau$ $(\tau_1-\tau_2)$ are greatly varied. When the $\Delta\tau$ for individual animals is examined it is found to be highly correlated with τ_1 (the correlation coefficient $= 0.76$ (Fig. 3)).

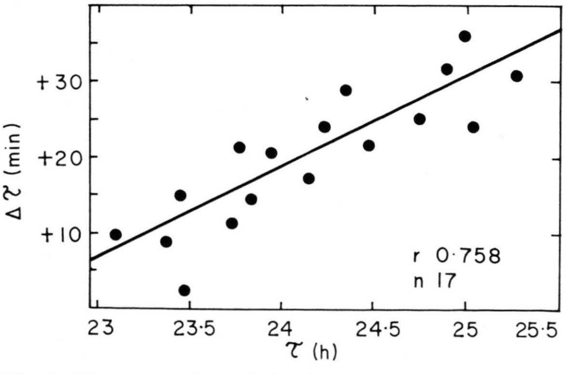

Fig. 3. The shortening of the free running period ($\Delta\tau$ in min) following Li$^+$ administration as a function of the initial period prior to Li$^+$ administration. Each point represents an individual animal. The straight line is the computed linear regression for the combined data (correlation coefficient = 0.76).

In most Li$^+$ treated animals duration of activity increases. This is apparent from the recordings in Fig. 2. In some cases the separation between activity and rest time becomes less clear. This was due mainly to activity at a "low level" during rest time.

Discussion

Li$^+$ offered through drinking water in bats had little or no effect on phase angle difference during entrainment. In contrast, Li$^+$ shortened the period length of the activity rhythm under free running conditions. The effect of Li$^+$ reported here differs from that previously found in other systems in which the period lengthened as a consequence to Li$^+$ ingestion [5, 6].

When the tenets of oscillation theory are applied to the biological rhythms, the prime generalization is that the conditions which influence the τ of the organisms influence also the phase angle (ψ) during entrainment. Such congruity of τ and ψ of the free running rhythm effected by light conditions were observed in birds [7] and in bats (R. Subbaraj, unpublished). However, Li^+ failed to exert such congruous action in entrainment. It seems possible that in the present experiment alternating steady state conditions such as DD/LL (on) and LL/DD (off) transitions under LD cycles [8] exerted a strong effect which inhibited the modulation of phase angle by Li^+. It is however conceivable that in a multi-oscillator and complex system such as this bat ψ may indeed change for another rhythmic parameter, for instance, the body temperature, which is not being monitored in our experiment.

An ample collection of free-runs ($n = 17$) in LL showed differences in their period lengths. Li^+ evoked smaller changes in τ in bats with shorter period length than in bats with longer period length. An explanation for similar phenomena in cockroaches by temperature changes was offered by Pittendrigh and Caldarola [8] which proclaimed that the τ variance is a homeostatic mechanism limiting the range of variation near lower/upper tolerated limits. Similar regularities are also detectable in our bats for Li^+. Such homeostasis of τ may be an offshoot of a more generalized integrity of the circadian rhythm which could be a functional prerequisite for an oscillator to serve the role of biological time measurement.

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