

**ARE LUNAR RHYTHMS A “ZEITGEBER”  
FOR CIRCUMNUTATIONS IN SUNFLOWER PLANTS  
(*HELIANTHUS ANNUUS*)?**

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Circumnutations are helical growth movements which are exhibited by most plants. This term is used to denote the rotary movements of plant organs in space (shoots, roots and tendrils), which occur in either an elliptical, circular or pendulum-like fashion.

The models of the circumnutation mechanism explain as both:

1. A combination of overcompensating reactions to gravitropic stimuli.

The gravitropic component of the circumnutation response is a result of gravitational and tidal forces. The bending rate is proportional to the gravitropic stimulation received by the hypocotyl. Gravity induces changes in membrane voltages, endogenous electric currents and ion fluxes. These changes point to the plasma membrane as the site of perception and transduction of the gravity signal. Gravity causes statolite translocation, which affects the state of ion channels and sets in motion calcium deposition. This leads to a reaction cascade connected with calmoduline activation. This stimulation cannot cause a reaction until the gravitropic reaction time has passed. Thus the system has a time lag which makes overshoots of the plumblin.

2. The result of endogenous oscillations.

Circumnutations are an example of circadian rhythms, which provide internal diurnal periodicity to anticipate daily changes in the plant's environment. Such rhythms are connected with a biological clock's network, which is predominantly based on transcriptional regulation. Such processes are autonomic, but can be modulated by self-sustained  $\text{Ca}^{2+}$  oscillation, growth hormone asymmetry and external stimuli such as light.

There are three diagnostic criteria that define a circadian rhythm:

- Persistence in constant conditions with an approximately 24h cycle
- Entrainment – sensing environmental time cues, such as light/dark (LD) cycles, and shifting the phase of the internal clock

- Temperature compensation – the cycle of the clock is sensitive to immediate temperature changes but is approximately the same at different constant ambient conditions

Our investigation aimed to verify the first criterion. We observed oscillatory movements in the hypocotyl of young sunflower (*Helianthus annuus*) plants, which are commonly used for studies of circumnutations. These movements were detected with a picture analysis system.

Self-sustained oscillations such as circumnutations are probably amplified by the successive gravitropic stimulations. So we assumed that when the growth conditions in a controlled environment reduce the interference of diurnal photoperiods (the lack of the main time marker - light/dark transitions) the rhythm of circumnutations will fit in match the lunar cycle. In fact, we observed only infradian rhythm corresponding to the phases of the Moon, although we expected a correlation between the daily rhythmic fluctuations in circumnutation and the timing and strength of the tides. However, the lunar rhythm of circumnutation activity was very clear. The activity peaks shown in the actograms do not cover the exact phases of the Moon, but it is known that even tidal reactions such as coastal tides reveal a time lag connected with features of the system. We noted that maximum activity correlates with extreme gravitropic stimuli, when the force is strongest or weakest (during the new and full moon as well as in the first and last quarter, respectively). The intermediate stages of the lunar cycle reflect the minimum of the activity fluctuations.

It is worth emphasizing that all the examined plants (except 1 of the 12) exhibited the same lunar rhythm of circumnutation activity. This means that the activity peaks occurred in the same stage of a lunar month. Plants germinated a few days later also fit in to the same lunar rhythm. It proves that activity fluctuations in LL are not related to the development rate, but to the lunar cycle, a secondary time marker. This rhythm may be connected with new leaf layers arising, but this demands further investigation.

We concluded that in constant conditions (LL) the rhythm of circumnutations does not transform to the circadian free running rhythm (FRR), but to the lunar rhythm, because of the lack of the primary time marker (*zeitgeber*) - LD alteration. In such conditions organisms match secondary time markers, such as match the secondary time marker - gravitational. Circadian FRR is probably a residuum of daily LD changes and has an exogenic origin. FRR conception, treated as a basic truth of chronobiology, could be a result of the investigation having been carried out for an insufficient period. We noted that the oscillation period is very variable in constant conditions. Thus, in the case of circumnutations, FRR, if present, has rather ultradian (about 2h) than circadian nature and reflects the rhythm of a single circumnutation.