A phase response curve for the locomotor activity rhythm of the rat

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Summary. A phase response curve was obtained for the locomotor activity rhythm of the rat, *Mus norvegicus albicus*, by interrupting a free-running rhythm with light signals of short duration. The response curve obtained was continuous and had the switch-over point at the position of 2 h before the acrophase, followed by the portion of advancing phase shift. The pattern of the curve was typical for the nocturnal rodent.

Entrainment of circadian rhythm to environmental light-dark cycles primarily depends on its periodically changing sensitivity to a light intensity, which is described in a phase response curve[1]. A number of response curves have been obtained in plants[2,3] and animals[4-7]. For rats, however, inspite of their frequent use in chronobiological researches, a phase response curve has not been published yet.

Methods. 4 rats of the Wistar strain, reared in a light controlled room (light/dark 12:12), were exposed to continuous light (5 Lux) at an age of 2 months. Spontaneous locomotor activity of the rats was monitored individually by a FARAD Animex Type S, which measured the changes in capacitance of a resonance circuit installed beneath a cage. The amount of activity was expressed by counts, which were recorded when the animal moved. A white light signal (800 Lux) of 60 min duration was given at different phases of the free-running rhythm once every 2 weeks. A phase shift induced by the light signal was measured in reference to an acrophase of the rhythm. Free-running periods and acrophases of the rhythms were calculated by a least squares spectrum analysis[8].

Results. Free-running periods of locomotor activity in 4 rats ranged from 24.4 to 24.9 h, but the periods were stable individually during the experiments. Figure 1 shows free-running rhythms of locomotor activity of 1 rat under dim illumination. A white light signal caused either a phase shift (left column in the figure) or a delay (middle column) or no shift (right column) depending on the phase used for the light signal. Apparently, shift of the onset of activity runs approximately parallel with that of the acrophase which was calculated by the least squares spectrum analysis. In the case of phase advance, a transient period was observed, taking several days to complete. In the phase delay, a phase shift occurred almost immediately without intervention of transient period. Figure 2 demonstrates a phase response curve constructed on the basis of the results obtained in figure 1. Phase shift was calculated from the shift of acrophase which was used for the phase reference in this study. The approximate position of the acrophase was at the midpoint of subjective night. The response curve was continuous. The portion of phase delay was observed in the early subjective night and the point of switch-over was detected at the position of about 2 h before the acrophase, followed by the portion of phase advance. The maximum effect of light signal in delaying the phase was about 2 h, and that in advancing it was about 4 h. Thus, the

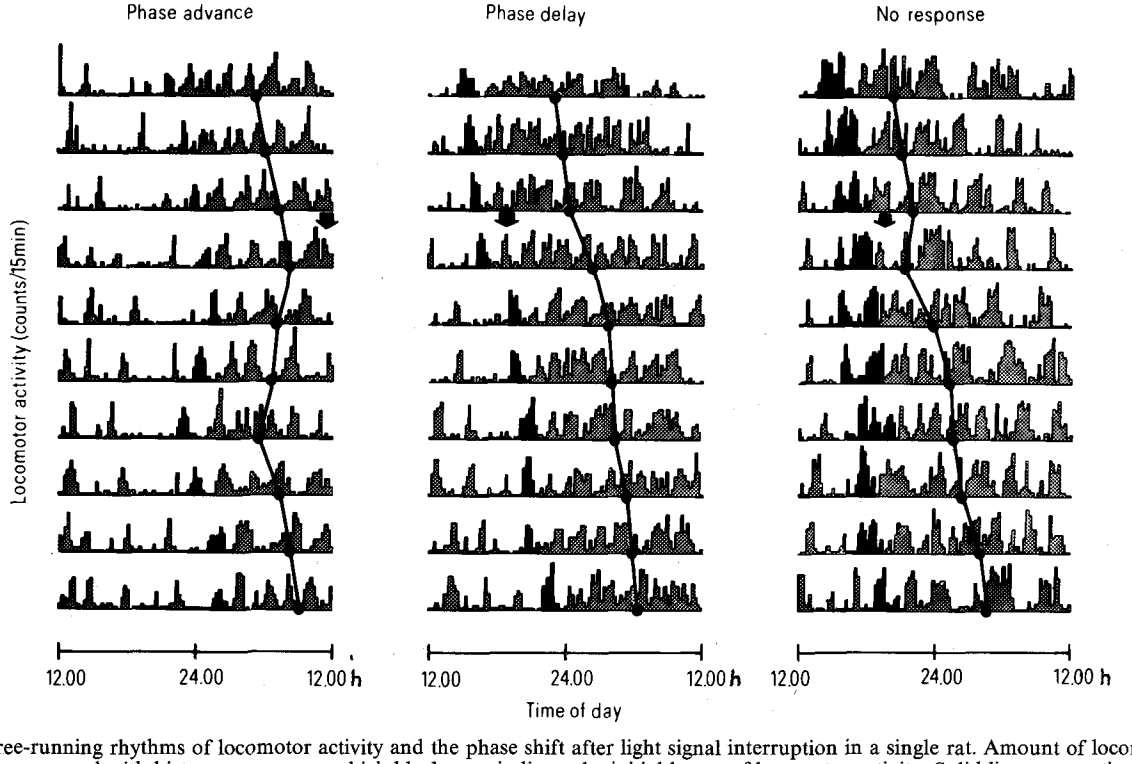

Fig. 1. Free-running rhythms of locomotor activity and the phase shift after light signal interruption in a single rat. Amount of locomotor activity is expressed with histograms, among which black ones indicate the initial bursts of locomotor activity. Solid lines connecting solid circles indicate shifts of acrophases for the purpose of easy visualization. In the left column, a phase advance of 3 h and 40 min was shown, as induced by a light signal which is indicated by an arrow. Transient period of the 1st 4 days was observed in this case. In the middle column, a phase delay (80 min) was obtained. No phase shift occurred in the right column, although slight deviation was seen in the 1st day after the signal. Free-running period of this particular rat was 24.9 h.

range of entrainment of the rhythm was estimated from the response curve to be from 21 to 27 h of light-dark cycles[9]. Individual differences in the phase response curve among 4 rats examined were small.

Discussion. Features of the present study, in which we obtained a phase response curve for the locomotor activity rhythm of the rat, are 2-fold: First, the instrument used in this study was to measure 'spontaneous' locomotor activity of the animal. In contrast, running wheel cages, previously used to obtain response curves in rodents[5-7], measured an activity accelerated by the wheel: i.e. rotation of the wheel itself affects an activity of the animal. Second, instead of the onset of activity, the acrophase of the rhythm calculated by the mathematical process was used as the phase reference point in this study. In this way, phase response curves were obtained even in rats which showed obscure onset of activity under free-running conditions. The extent of the phase shift, estimated by a difference of acrophases, reflects the changes of both phase and activity amount induced by a light signal.

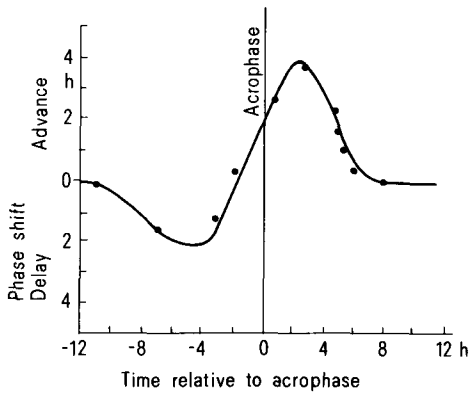

Fig. 2. A phase response curve for locomotor activity of a rat. Phases of the rhythm are expressed by the time relative to the acrophase which was located approximately at the midpoint of subjective night. The acrophase is indicated by a perpendicular line at the point of zero phase (a phase reference point). Before the acrophase, phases are indicated by negative sign and after the acrophase by positive sign. The point of switch-over is detected at about -2 h. The response curve is characterized by 2 distinct portions of phase delay and phase advance: the phase delay was observed in the early subjective night, followed by the phase advance. The maximum phase advancing effect of light signal (advance of about 4 h) was located 2 h after the acrophase.

The phase response curve obtained in the rat was very similar to those obtained in deer mice[5], flying squirrel[6] and hamster[7]. Intensity of the background illumination, intensity and duration of the light signal or kind of method used to obtain the response curve, all these factors were reported to affect the shape and the size of the phase response curve[1]. Nevertheless, essentially the same pattern of the response curve obtained in nocturnal rodents suggests an operation of a common phase control system in these species.

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Ca-electrogenesis in mealworm muscle: A voltage clamp study

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Summary. The membrane current in the muscle fibre of larval mealworm, *Tenebrio molitor*, was characterized by an early transient inward current followed by a late outward current. The results suggest that the inward current is associated with an inward movement of Ca ions down its electrochemical gradient, and Na ions have little to contribute to the inward current in this fibre.

During the past 6 years, substantial evidence has accumulated that Ca ion is the charge carrier of inward current at the non-synaptic membrane of insect skeletal muscle fibres[3-9]. One of the major paradoxes in the acceptance of this evidence is the reported Na-dependent electrogenesis in the muscle fibres of larval mealworm, *Tenebrio molitor*[10,11], in which increased Ca concentration of external medium depressed the directly evoked muscle spikes[12]. We wished to determine whether or not this particular ionic permeation of *Tenebrio* muscle fibre reflected a true phylogenic specialization. Here we report the use of the voltage clamp technique to solve this problem on ionic requirements for the electrogenesis in *Tenebrio* muscle. There has been no previous report on voltage clamp analysis of non-synaptic membrane in insect muscle fibres, except the preliminary note on TEA-treated locust muscle fibres[13].

We studied the segmental muscle of the body wall of immature larvae of *Tenebrio molitor*. The muscle fibres were clamped using a 2-microelectrode method, with a potential recording electrode connected to a current passing electrode through a high input impedance amplifier and a feedback amplifier (Dagan model 8500, Minneapolis, Minn.). The short muscle fibres, which have a length less than 800 µm and a diameter of about 100 µm, were used in the experiments to establish a good condition for space-clamp. A mean value for the length constant using the short cable model[14], was 1.4 mm. Thus, the efficiency of the clamp was greater than 96%.

Although many insect muscle fibres cannot generate all-or-none action potentials in normal saline, *Tenebrio* muscle fibres responded to an outward current pulse with a spike in normal saline (NaCl 70 mM, KCl 30 mM, $CaCl_2$