

The values of heart rate recorded from the monitoring rate meter were rechecked from the sinus complexes of the ECG. These values, therefore, represent the pacemaker frequency.

Discussion. In the isolated denervated hearts, extrinsic neurohumoral influences are absent. Any possible thermal or chemical effect was also ruled out because the perfusing and distending solutions had the same temperature and chemical composition. Further the rate changes only occurred during change in the pressure without any other variable being involved, and the rate changes were quite reversible on reversing the pressure change. Therefore, the observed chronotropic changes due to alterations in the transmural pressure could only be attributed to changes in the pacemaker activity in response to pressure-induced mechanical effect.

The present results are quite consistent with the previous observations on pacemaker response to mechanical stretch (PATHAK¹⁻⁴; PATHAK, JOG and GOYAL⁷). In previous approaches to the assessment of basic intrinsic heart rate (JOSE and COLLISON⁸), the role of pressure-induced mechanical stretch was not appreciated. The present work establishes that the true basic intrinsic heart rate can only be obtained when, besides eliminating the extrinsic neurohumoral and thermal influences, the effect of pressure induced stretch is also excluded. The minimum heart rate at 0 transmural pressure, therefore, represents the true basic intrinsic pacemaker frequency. It is also clear that the transmural pressure is an important determinant of basic intrinsic heart rate.

⁸ A. D. JOSE and D. R. COLLISON, *Cardiovasc. Res.* 4, 160 (1970).

Rudiments of an Ability for Time Measurement in the Cavernicolous Fish *Anoptichthys jordani* Hubbs and Innes (Pisces Characidae)

WILHELMINE ERCKENS and F. WEBER

Zoologisches Institut der Universität, Badestrasse 9, D-44 Münster-Westfalen (Federal Republic of Germany), 6 April 1976.

Summary. Rudiments of an ability for endogenous time-measuring are indicated a) by bimodal activity in the dark phase of LD-cycles of 16:16 or 24:24 h and b) by damped activity oscillations frequently following a transition from LD to constant conditions. These oscillations always have the same period length as the applied LD.

One may expect that the circadian rhythm depending on the integrity of many genes does not disappear suddenly but degenerates gradually during the regressive evolution under cave conditions. The knowledge of these degeneration steps may allow conclusions on genetics and physiology of the intact endogenous clock. The cavernicolous fish *Anoptichthys jordani* is a suitable object for such investigations, because it is genetically connected by intermediate forms with its surface ancestor *Astyanax mexicanus*¹. This work is concerned with investigations of an endogenous stochastic control and of rudiments of an endogenous rhythm of swimming activity in 4 specimens (3 ♂♂, 1 ♀) of the blind extreme cavernicolous form.

Methods. The swimming activity was recorded by infrared beams fixed 2–3 cm below the water surface (in some experiments an additional beam near the bottom).

The measuring interval was 30 min. The animals were isolated from each other during the experiments. According to the length of the artificial light period, the LD experiments lasted 215–606 h. The DD and LL experiments lasted 304–316 h. The series of data were investigated with regard to periodic distributions by a periodogram analysis program² in the computer-centre of the University of Münster, and with regard to stochastic distributions of activity by the methods applied by LEHMAN et al.³.

¹ C. M. BREDER, JR., *Zoologica* 27, 7 (1942).

² G. LAMPRECHT and F. WEBER, *Pflügers Arch.* 315, 262 (1970).

³ U. LEHMANN, D. NEUMANN and H. KAISER, *J. comp. Physiol.* 91, 187 (1974).

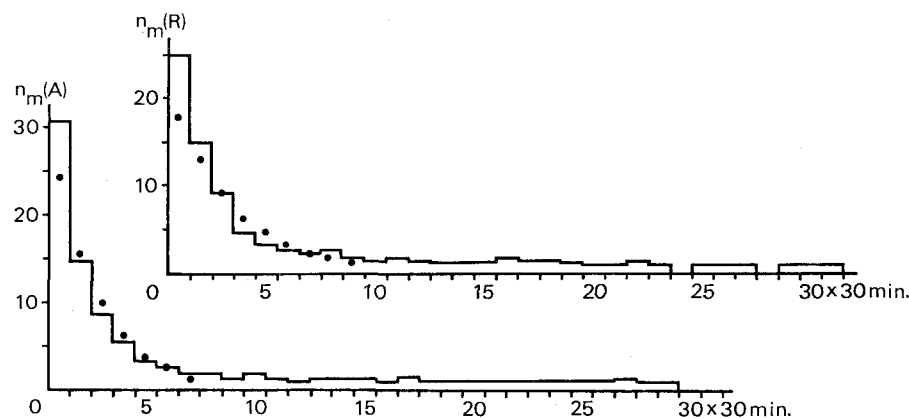


Fig. 1. Geometric means of frequency histograms of A and R from 20 DD experiments. Ordinate: means of the observed values. Points: fitted exponential function in the range of agreement including 71% of the A- and 70% of the R-values (χ^2 -test, $p_A = 0.88$, $p_R = 0.75$. $f_A = -0.48$, $f_R = -0.34$).

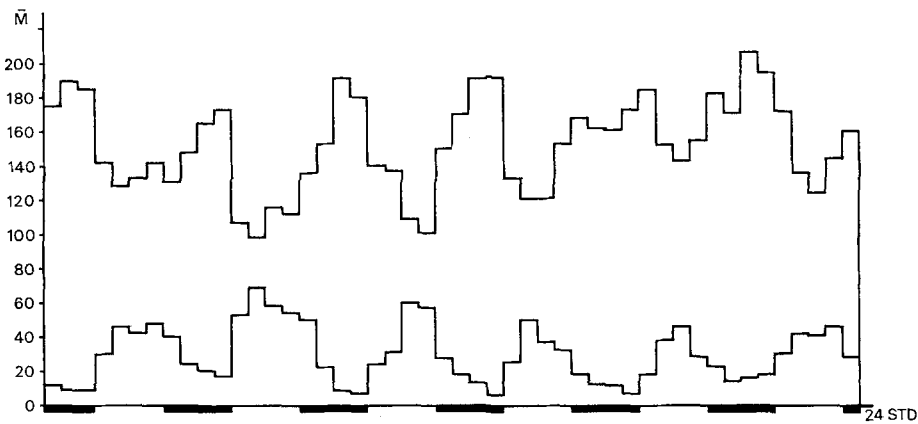


Fig. 2. Surface and bottom activity of a fish in a LD of 2:2 h. Abscissa: hours; ordinate: mean activity. Blackbeams: darkphases.

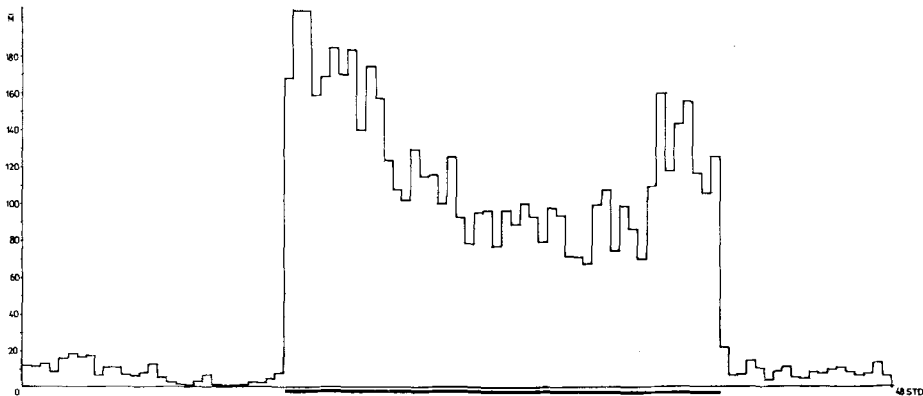


Fig. 3. A bimodal activity pattern in a LD of 24:24 h. For symbols see Figure 2.

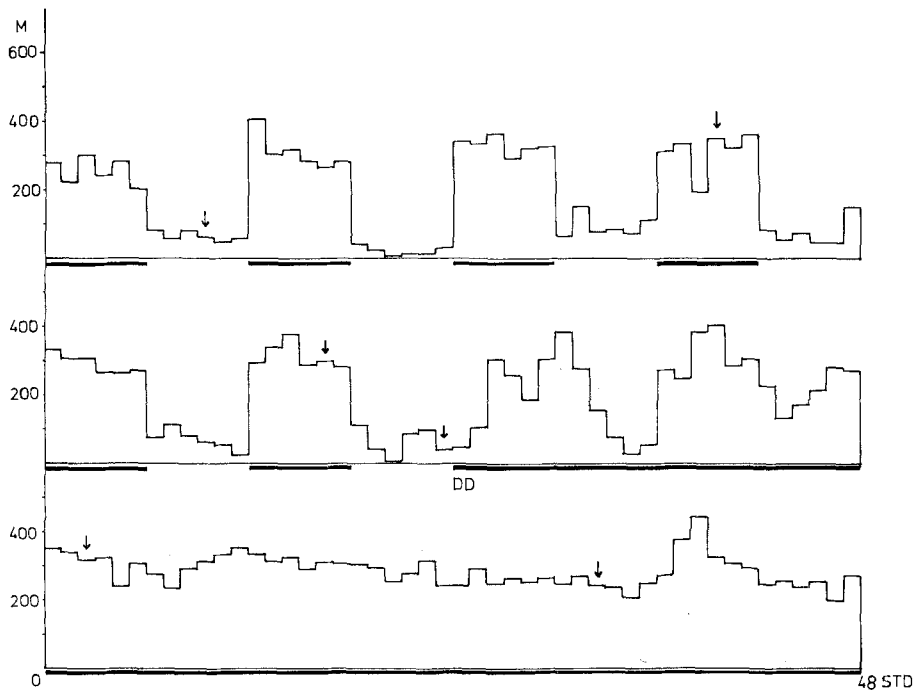


Fig. 4. 2 'postoscillations' after transition from a LD of 6:6 h to DD. After that the typical DD behaviour. Abscissa: hours; ordinate: activity amounts of 30 min; arrows: automatic feeding. Black beams: dark phases.

Number of ‘postoscillations’ after the transition from LD to DD or LL

| Animal | DD after LD | | | | | | | | LL after LD (1 Lux) | |
|--------|-------------|-----|-----|-----|-------|-------|-------|-------|---------------------|-------|
| | 2:2 | 3:3 | 6:6 | 8:8 | 12:12 | 12:12 | 16:16 | 24:24 | 12:12 | 12:12 |
| 1 | / | 1 | 2 | 1 | 1 | (1) | 1 | – | / | 5 |
| 2 | / | (1) | 4 | 2 | 1 | 1 | – | – | 2 | – |
| 3 | 1 | (1) | (1) | 1 | 3 | 2 | 1 | – | / | – |
| 4 | 1 | – | 1 | 1 | 4 | – | – | – | – | – |

–, no postoscillation; (), weak postoscillation; /, selenium cell defect.

Results. The periodogram analysis of DD and LL experiments (1.0 Lux) showed neither free running circadian nor infradian or ultradian rhythms. The animals were completely arrhythmic and permanently active without resting. To distinguish between relative activity and relative resting, the mean of the 30 min data was defined as a threshold. The duration of the activity time above the threshold (A) was not correlated with the duration of the following or of the preceding activity time below the threshold (R). The frequency histograms of A and R could be approximated by negative exponential functions of the type $y = a \cdot e^{-f \cdot x}$ (at least in the range of classes with short and medium durations). The exponential range of the average frequency histogram of A enclosed 71%, that of R enclosed 70% of all values (Figure 1).

In all applied LDs [2:2, 3:3, 6:6, 8:8, 12:12 (5 × each), 16:16, 24:24 h, 20 or 1 Lux in the light phase] 3 animals showed rhythmic activity patterns with the period of the corresponding LD. The 4th animal was aperiodic in 2 LDs of 12:12 h. The selenium cells near the water surface always recorded more activity in the dark phases than in the light phases, whereas near the bottom the activity showed either aperiodic distributions or maxima in the light phases (Figure 2).

In the LDs of 24:24 and 16:16 h, the activity of all the animals decreased in the middle and increased again at the end of the dark phases (Figure 3); 1 animal also showed such bimodal activity in the dark phases of the LDs of 6:6, 8:8 and 12:12 h.

After a transition from a LD of 6:6, 8:8 or 12:12 h to DD conditions, the swimming activity frequently oscillated 1 to 4 times with the period of the preceding LD (Figure 4 and Table). After that the typical behaviour under DD conditions was observed.

Discussion. Permanent and aperiodic swimming activity is also known of other cavernicolous blind fish species⁴. Because of the absence of endogenous rhythmic components the existence of stochastic basic mechanisms controlling the swimming activity can be proved³. This proof is only possible on the condition that during the experiments the probabilities of transitions between the two different activity levels remain unchanged. Whether this assumption is exactly valid for *A. jordani* is unknown. The transition probabilities are time-independent in the range of short and medium durations of the two distinguished activity levels. It may be concluded that the transitions are controlled by endogenous stochastic processes. Such controlling mechanisms are at first found in *Uca* species^{3,5}.

Our induction experiments with LDs of different period lengths verify the ability of *A. jordani* to perceive light and adapt its activity to LD periods, even to periods with a small intensity amplitude. The light receptors are probably localized in the brain⁶.

The activity behaviour after a transition from LD to DD indicates rudiments of an ability for measuring time. The underlying physiological mechanisms should be fit for damped oscillations. The ‘postoscillations’ lasting for several periods can hardly be explained by another model.

The bimodal activity patterns also point to the rudimentary ability for time measuring. The observation that the bimodal pattern can also appear in relatively short LDs (for example 6:6 h) is contrary to the assumption that the activity minima in the middle of the dark phase are caused by physiological exhaustion. The bimodal patterns agree with the phenomenon of the ‘postoscillations’ in so far as the endogenously measured time interval does not depend on a circadian clock but on the period length of the applied LD.

The cavernicolous species *Chologaster agassizi*⁴ and *Anoptichthys antrobius*⁷ react to light like *A. jordani*. Possibly they are also endowed with rudiments of an endogenous time-measuring mechanism. *Typhlichthys osborni* and *Anoptichthys hubbsi*, which react indifferently to light^{8,9}, have probably lost this ability completely.

⁴ G. THINÉS, *L'évolution régressive des poissons cavernicoles et abyssaux* (Masson et Cie., Paris 1969).
⁵ H. KAISER and U. LEHMANN, *J. comp. Physiol.* 96, 1 (1975).
⁶ J. KÄHLING, *Biol. Zentbl.* 80, 439 (1961).
⁷ G. THINÉS and F. WOLFF-VAN ERMENGEN, *Anim. Behav.* 13, 585 (1965).
⁸ M. L. VERRIER, *Bull. Mus. Hist. nat.* I, 1, 82 (1929).
⁹ C. M. BREDER, JR., and P. RASQUIN, *Bull. Am. Mus. nat. Hist.* 89, 323 (1947).