Exogenous and Endogenous Control of Swimming Activity in *Astyanax mexicanus* (Characidae, Pisces) by Direct Light Response and by a Circadian Oscillator

II. Features of Time-Controlled Behaviour of a Cave Population and their Comparison to a Epigean Ancestral Form

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Z. Naturforsch. **37 c,** 1266–1273 (1982); received August 16, 1982

Astyanax, Cave Fish, Regressive Evolution, Circadian, Light Response

1. The swimming activity of 6 specimens of the Pachon cave form of *Astyanax mexicanus* was tested with regard to its time control under various light-dark(LD)cycles and constant conditions, and it is compared to that of a river form.

2. In general, activity is entrainable by all applied LDs, but even if the amplitude of a forcing signal increases the signal energies are lower than in the river fish.

3. In case of entrainment the maximum values of surface activity correspond to the dark phases, those of bottom activity to the light phases of a LD. Flexible patterns – as often observed in the river form in the range of resonance about 24 h – are very seldom. Furthermore, disturbances often occur in the entrainment of one activity form, or one form runs arrhythmic while the other is still entrained.

4. The activity answers to changing environmental conditions are not as uniformly quick as in the river fish. But the system hardly needs a swing-in time to become entrained when a LD starts.

5. After transition from LD to DD (=constant darkness) the entrained rhythms disappear immediately.

6. In no LD with a period length differing from 24 h a circadian rhythm can be observed in addition to the entrained frequency.

7. These results show that the passive system of the river form has developped into an extremely passive one being unable to oscillate and thus has become simplified during regressive evolution. Concerning the circadian oscillator of the epigean ancestor, it was also subjected to regression, but it has not been completely lost. After a LD with a period length about 24 h the circadian oscillator is able to act as a stable system, clearly shown by the freerunning circadian rhythms of surface activity. But out of this range the oscillator is unable to control activity. In DD after all other LDs activity patterns are arrhythmic.

Introduction

The epigean river fish *Astyanax mexicanus* is the ancestor of cave populations inhabiting a large number of caves in Mexico. This is one of the very few cases in which the epigean ancestral form of a cave animal is recent, and both forms – epigean as well as hypogean – belong to one and the same species. The attractivness of *Astyanax* in research is due to this reason for the most part. Therefore, a large number of papers are dealing with its morphology, ethology and genetics (review: Wiley and Mitchell [1]) in order to work on the numerous implications this species offers with regard to a better understanding of evolutionary processes during adaptation to underground environments. In the field of its time-controlled behaviour our knowledge is incomplete and should be advanced since the existence of a circadian oscillator in the epigean ancestor is proved (Erckens and Martin [2]).

The cave fish of the Cueva de El Pachon was first described by Alvarez [3] as "*Anoptichthys antrobius*". The Pachon-population is among those being extremely reduced, though there are no hints of an important gradient among the different cave populations in the number of degenerated genes concerning the regression of eyes and pigment (Wilkens [4]). There is one exception: the Micos form, which is regarded as a phylogenetically young cave fish, so to speak in "statu nascendi" (Wilkens and Burns [5]; Wilkens [6]). Therefore, its circadian behaviour

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would be of great interest when compared to the epigean river form and the extremely cave adapted Pachon form (Erckens and Martin [7]). As already pointed out, little is known about the time-control systems in the cave fish. Thinès *et al.* [8] report that no clearcut rhythms appear in the activity of "*Anoptichthys antrobius*" neither under natural light-dark-cycles nor under constant conditions. A lot of papers, which emphasize a still existent ability of light perception, are dealing mostly with the Chica form "*Anoptichthys jordani*", which probably is a hybrid population. Therefore, it would be better to attach no great significance to these results because this population is not representative for the situation in true cave animals (Mitchell *et al.* [9]). Recently, Thinès and Weyers [10] report on their research concerning the cave fish "*Astyanax jordani*" (the Chica form? Unfortunately, the authors give no precise information about the origin of this cave fish). "*Astyanax jordani*" is entrainable by light-dark-cycles, though the entrainment is not very stable. In the course of the LD-experiments the activity becomes arrhythmic in most cases. Under constant conditions no significant rhythms appear.

Three questions should be answered in this paper: 1. Is the Pachon cave form still sensitive to light stimuli? 2. If the activity is entrainable by light-dark-cycles in which way will the external controlled system have changed its properties during regressive evolution? 3. Does there still exist a control of a circadian oscillator on activity?

Materials and Methods

Six adult specimens (♂ and ♀) of a F_1-inbreeding of individuals caught in the Cueva de El Pachon (Sierra de El Abra, Mexico) in 1975 were available (for detailed information about breeding see Wilkens [4]). Pigmentation and eyes were reduced.

The techniques in collecting data, applying forcing and constant conditions, in IR-video observations and in mathematical analyses were the same as described by Erckens and Martin [2]. There are two exceptions: 1. LDs with harmonics of 24 h as period lengths were not applied. 2. The amplitude of illumination was increased. During the light phases illumination was about 190 lux on a average in contrast to 1 lux in the river fish tests. During the dark phases light was switched off. Concerning the IR-video observations the behaviour of the cave fish is classified into 3 components only:

1. swimming near the surface;
2. swimming near the bottom;
3. swimming up and down the pane.

Results

IR-video-observations

The behaviour of the Pachon cave fish can be classified into three components being equivalent to the same components of the river fish. In difference, the cave fish hardly shows the component "resting near the bottom", but it is possible to distinguish between different speeds in its permanent swimming. Fig. 1a presents the results of observations in a LD 6:6 h. The component 1 exhibits no large amplitude, but it reaches its maximum obviously in the dark phase of the LD. The course of component 2 is inverse and exhibits its maximum values in the light phase. The component 3 mainly appears in the ranges of illumination changes. When reduced to the two components SURFACE (comp. 1 + 1/2 comp. 3) and BOTTOM (comp. 2 and 1/2 comp. 3), two inverse oscillations with low amplitudes result (Fig. 1b). Their phase relations are the same as in the river fish: surface activity with a maximum in the dark phase and bottom activity with a maximum in the light phase of the LD. Thus, it seems that the special experimental arrangement, which was already used in the river fish tests, was also suitable to analyse the behaviour of the cave fish.

Analyses of time series

General remarks

Some general remarks should be made. Under entrainment, the cave fish shows a tendency to build up the same inverse activity patterns as observed in the river fish (Fig. 2). But flexible reactions as reported on the river fish are extremly rare. There is no important difference between the levels of both activity forms: on an average, bottom activity is slightly higher than surface activity. The latter is tending to change its level but without coherence to the forcing signal. Only in very few cases, the maxima of activity are clearcut. Normally, they are displayed against a high level of background noise (Fig. 4). Very seldom, the actograms show an alteration of sections with activity and sections without activity. Maxima and minima normally are expressions of the instantaneous level of permanent activity.

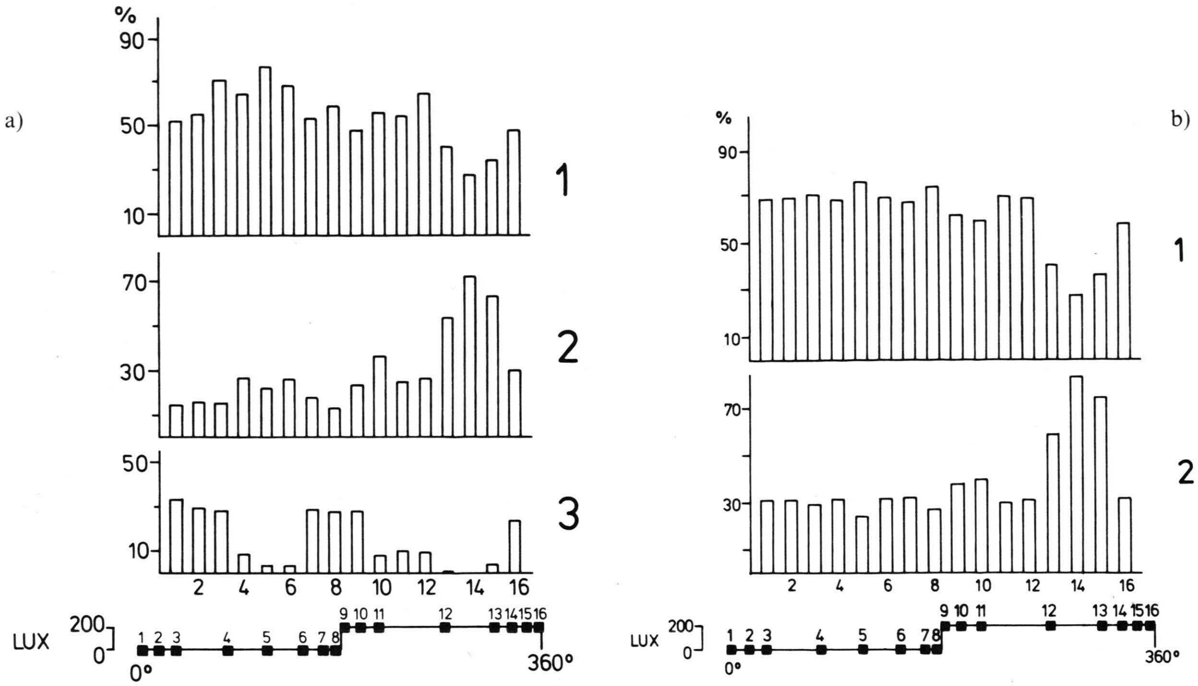

Fig. 1. Distribution of behavioural components of a specimen in a LD 6:6 h. Abscissa: number of observations (1−16); ordinate: percentual amount of the components in comparison to the total observation time (100%). Below the histograms the LD (200−0 lux) is marked as a rectangular signal. Black bars indicate positions and lengths of the 16 observations. a) Distribution of each single component: 1. Swimming near surface; 2. Swimming near bottom; 3. Swimming up and down the pane. b) These three components are reduced to: 1. SURFACE (comp. 1+0.5 comp. 3) and 2. BOTTOM (comp. 2+0.5 comp. 3).

Mathematical analyses

The activity can be entrained by all applied LDs (Fig. 3), but a few restrictions must be mentioned. In a LD 12:12 h with an amplitude of 1 lux (the same as applied in the river fish tests) an undisturbed entrained signal rarely occurs (Fig. 4a, b). The bottom activity mainly shows a stronger entrainment though with a low amplitude. To stabilize the forced signal, it is necessary to increase the amplitude of the forcing signal (Fig. 4a, b). Therefore, illumination during the light phases of all further LDs was enhanced to nearly 200 lux. Nevertheless, the forced signals exhibit low and modulated amplitudes and slightly shifting phases (Fig. 4a, b). Consequently, the signal energies of the average signals are generally low. The averaged maximum values are about 24%, the minimum values about 7% (Table I). In case of lowest values, the activity can be considered as arrhythmic. In these cases, there are of course no longer inverse patterns, but one activity form is always entrained while the other one is not. Arrhythmic patterns do not occur every time during the entire course of the

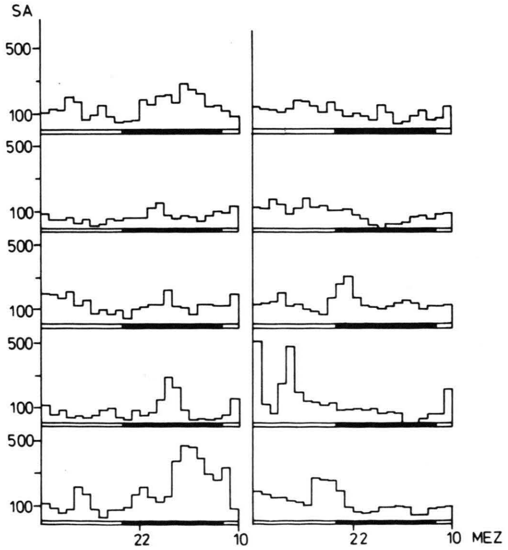

Fig. 2. Surface-(left) and bottom activity (right) of a specimen during 5 days in a LD 12:12 h. Abscissa: time scale (clocktime: MEZ); ordinate: amount of swimming activity per hour. The maximum values of surface activity mainly correspond to the dark phases, those of bottom activity in most parts to the light phases of the LD, but without being marked as well as in the river fish.

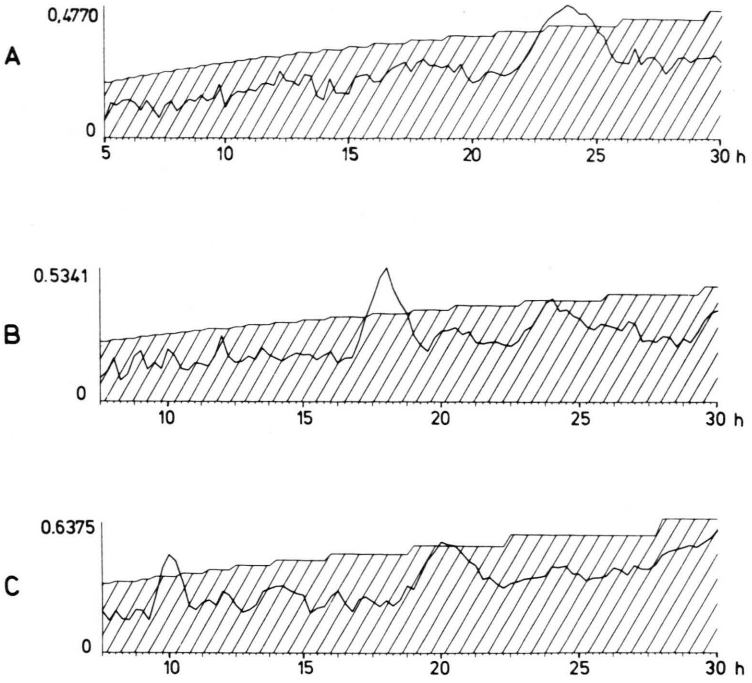

Fig. 3. Periodogram analyses of three time series of surface activity. A: LD 12:12 h; B: LD 9:9 h; C: LD 3:7 h. The peaks beyond the shaded areas indicate significant rhythms (entrainment of oscillations); test niveau is 95%.

Table I. Signal energies of average signals of surface-(SA) and bottom activity (BA) computed at the period length (in h) of each LD. The percentual amount of the signal in relation to the total energy amount of the process (100%) is represented. $\overline{SA}$, $\overline{BA}$: averaged values; –: no registration; P1 – P6: 6 different specimens of the Pachon cave population; LD 12:12 I: LD with low illumination amplitude (1 – 0 lux); LD 12:12 II: LD with high illumination amplitude (about 200 – 0 lux).

		12:12 I	12:12 II	8:8	9:9	3:7	16:16
P1	SA	14.2	22.4	11.3	2.9	2.1	5.1
	BA	19.2	23.3	11.5	18.3	8.9	13.0
P2	SA	7.5	15.9	11.8	10.5	6.5	4.0
	BA	14.5	25.5	30.8	12.9	7.9	6.0
P3	SA	14.9	21.7	–	48.3	8.9	41.0
	BA	26.8	17.3	12.2	26.5	8.2	17.5
P4	SA	12.2	37.4	4.0	9.6	14.7	6.4
	BA	13.1	15.3	9.3	–	–	–
P5	SA	10.1	11.0	33.0	9.1	6.1	10.8
	BA	11.8	7.7	6.0	5.4	2.0	6.7
P6	SA	18.9	19.2	13.5	29.3	3.0	16.9
	BA	7.3	53.7	14.3	–	–	10.8
	$\overline{SA}$	12.9	21.3	14.7	18.3	6.9	14.0
	$\overline{BA}$	15.5	23.7	14.0	15.5	6.8	10.8

time series, but sometimes only in parts of the series, interrupted by parts in which an entrainment is detectable.

The oscillation of total activity normally looks unentrained because of the inversity of the two single oscillations, which are, moreover, comparable in the strength of their entrainment (Fig. 4c). Therefore, no form dominates clearly, and the summing-up of both results in this particular pattern.

The reactions of the forced signals on the external stimuli are not uniformly quick. There are time series showing reactions within the first measuring interval of 1/2 h and others where reactions do not occur before several hours have passed. After a transition from LD- to DD-conditions the entrained frequencies die away at once.

The question whether the time series of LD-tests contain circadian rhythms in addition to the entrained frequencies has to be denied.

Frequency analyses of DD-data nearly lead to the same result, but there are two important exceptions. That means, the activity in DD is arrhythmic in most cases, but in DD after a LD 12:12 h the surface activity exhibits a freerunning rhythm with a low amplitude and a slightly shifting phase (Fig. 5), where-

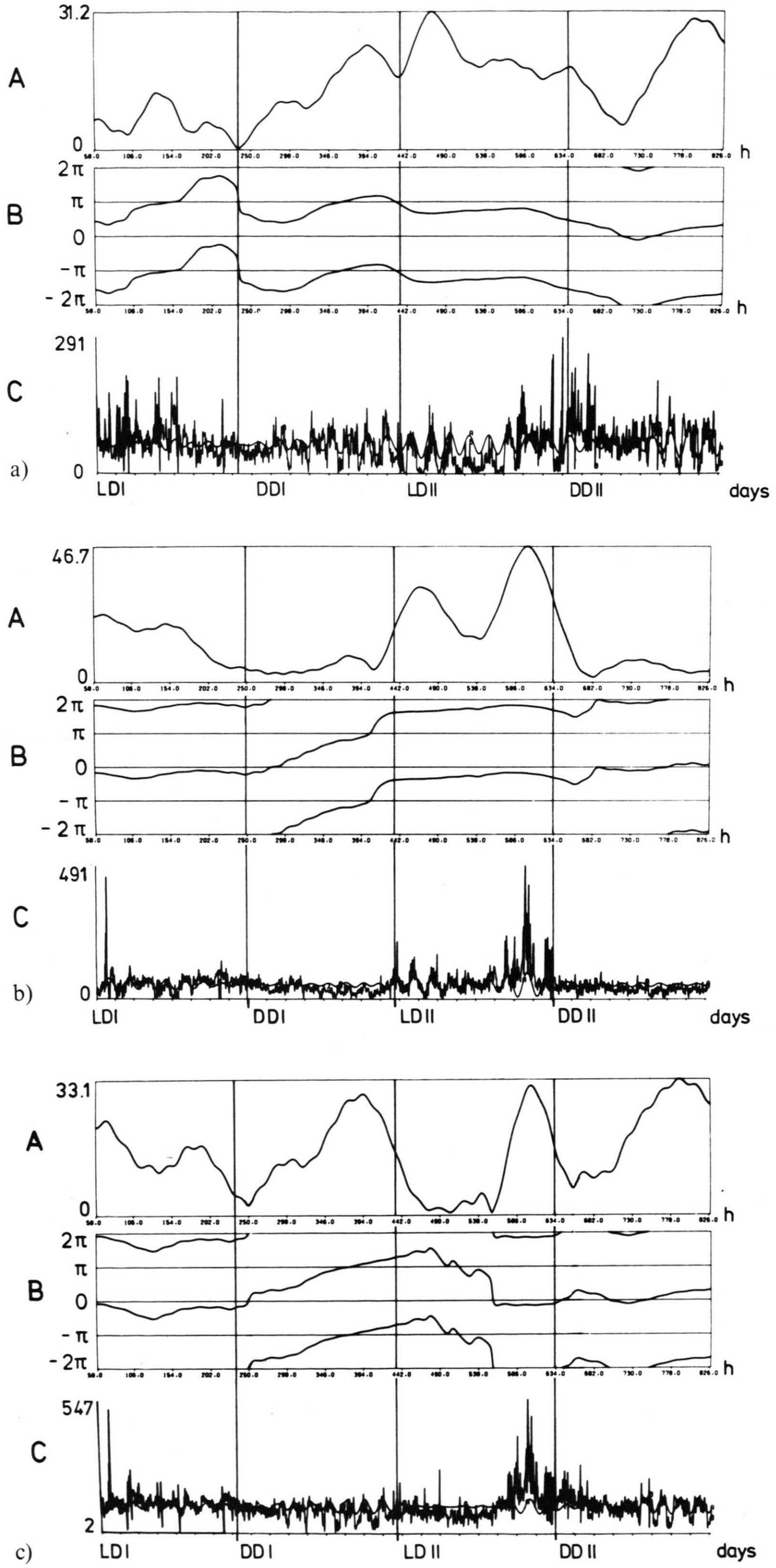

Fig. 4. Complex demodulation of time series of surface- (Fig. 4a), bottom- (Fig. 4b) and total activity (Fig. 4c) in a LD 12:12 h with low illumination amplitude (LD I, 1−0 lux) a following DD (DD I) and in a subsequent LD 12:12 h with an increased illumination amplitude (LD II, 200−0 lux) and a following DD (DD II). A: estimated instantaneous amplitude; B: estimated instantaneous phase; C: observed data and remodulated signal; demodulation period: 24 h; band pass filter obtained by windowing the truncated impulse response of the desired ideal pass-/stopfilter by the Kaiser-Bessel window; right passband edge at 22.00 h^{-1}; stopband starts at 20.75 h^{-1}; ordinate values in A and C: number of impulses per 1 h. The entrainment of surface and bottom activity is stronger in case of high illumination amplitude. In both DDs surface activity is freerunning, whereas bottom activity does not show a significant rhythm. The oscillation of total activity is not strongly entrained in LDs because of the inversity of both single oscillations. Its rhythm becomes significant in DD because surface activity is now dominant.

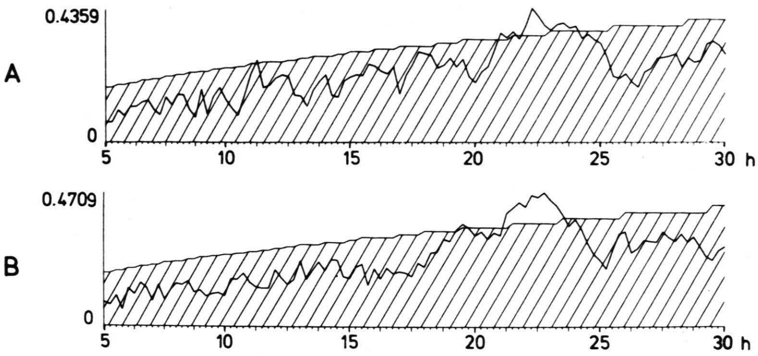

Fig. 5. Periodogram analyses of two times series of surface activity in DD after LD 12:12 h (above) and after LD 8:8 h (below). The peaks beyond the shaded areas indicate freerunning rhythms (22.2 h above; 22.5 h below); test niveau is 95%.

as the bottom activity normally runs arrhythmic. The second exception is observed in DD after a LD 8:8 h. In half the cases a freerunning rhythm swings in the surface activity as after release from LD 12:12 h.

Discussion

The cave populations of *A. mexicanus* probably became separated from epigean populations in the early pleistocene (Myers [11]). During their regressive evolution they have developped several attributes which are typical of cave animals; the most obvious are regressions of eyes and melanophore system. The basic genetical mechanisms of the evolutionary processes which took and still are taking place in the course of advancing adaptation to cave environments are items of many efforts in research. Probably, the time-measuring systems of the epigean river fish were involved in this regression. In underground habitats the strongest forcing signals as LD and daily changes of temperature are absent since a long time, and one should suggest that these facts must have an influence on the stability of the time-controlled systems.

The Pachon cave form is still reacting on light stimuli though it is less sensitive than its epigean ancestor. The lower sensitiveness is not astonishing at all because the cave fish has lost its visual information input. This input seems to play an important role in the perception of light stimuli being able to entrain activity. This is suggested by the observation that after loss of eyes in the cave fish it is necessary to increase strongly the amplitude of the forcing signal in order to stabilize the forced oscillation. But obviously, eyes cannot be the only receptors for light as a forcing signal. An ability of light perception not depending on retinal structures seems to be widespread in Teleostei (de la Motte [12]) and depends often on pineal light receptors. Since these receptors are reduced in *Astyanax* (Hertwig [13]) it is not yet clear in which way light as a forcing signal is perceived.

The Pachon cave fish exhibits a permanent activity, but different speeds can be observed. Real resting is extremely rare. Under light conditions the greater part of activity takes place near the bottom, but not in a comparable extreme way as in the river fish. Thus, the "photonegative" reactions of the epigean fish still exist, but they are not marked as well. In general, the swimming behaviour of the cave fish is very similar to that of the river fish in darkness.

The cave fish actograms exhibit in general the same inversity of the two activity forms as reported in the river fish though some restrictions have to be made. There is a greater variability in activity answers to external light stimuli as in the river fish. This variability mainly means a weaker entrainment at least in parts of a time series, sometimes during the entire course of the test. That is very different from the river fish, which always exhibits a strong entrainment of both activity forms. Weak, resp. strong entrainment are measured by the energy of the average signal of the entrained oscillation. The flexibility of phase relation is observed very seldom, thus, it seems to be a feature of river fish's activity. In case of entrainment both activity forms mainly keep their well-known phase relation. This uniformity of patterns is similar to that of the river fish out of the range of resonance. The oscillations of both activity forms are nearly of the same strength. Therefore, when added up the resulting oscillation hardly exhibits an entrainment. In this case, it is much more important than in the river fish to analyse surface and bottom activity separately and not total activity alone; otherwise one could conclude that the cave fish is not entrainable at all by LDs.

The passive system

Obviously, the activity of the Pachon cave fish is under control of a passive system with a nearly unlimited range of entrainment and a linear relationship between input and output. Features of the entrained rhythms are modulations of their instantaneous amplitudes and shifts of their instantaneous phases. The signal energies make obvious that the stability of the forced oscillations has decreased when compared to the river fish. In general, the damping of the system seems to be unchanged. The entrained rhythms disappear at once after transition from LD to DD. That means, the system is unable to oscillate and is, therefore, extremely passive. The passive system of the river fish being able to oscillate is of a more complicated structure and is to regard as "higher evolved". Röhler [14] emphasizes that an extremely passive system can be developped out of a passive one of the same structure if feedbacks fail. The speculation is striking whether the lack of feedback from eyes to brain in the cave fish gave reason for the simplification of the system.

The selfsustained circadian system

Has the circadian oscillator of the river fish lost its control on activity during advancing adaptation to cave environments? At first sight it looks as if this is true. No oscillations with period lengths in the circadian range are detectable in addition to the entrained frequencies under forcing conditions. The frequency analyses of most DD-data also seem to prove a lack of circadian control because no significant rhythms appear. Actually, the situation is more complicated. Important exceptions from these results are observed in DD after LD 12:12 h and in a weaker form in DD after LD 8:8 h. In these particular cases, at least one activity form exhibits a clearcut freerunning rhythm with features comparable to the rhythm under synchronizing conditions. Freerunning rhythms demand a selfsustained circadian oscillator as an underlying system. So, it seems that the Pachon cave form has kept an oscillator with a restricted ability of selfsustenance. Only under special forcing conditions, *i. e.* in the range of period lengths about 24 h the oscillator is able to act as a stable system and to remain stable under following constant conditions. Out of this range the oscillator seems to remain switched off, and the activity only shows the entrained rhythms under forcing conditions and arrhythmic patterns under constant conditions.

The lasting existence of this oscillator is probably a relic. The processes of its regression are still poorly known, but it might be useful for understanding to regard further regressive processes in *A. mexicanus* and other cave fishes concerning the genetics of eye and pigment. These structures are complex networks of different components and their developments and functions base on internal correlations. If such systems are changed by regression during advancing adaptation to cave environments they disappear gradually in a quantitative way; never exist fragments (Wilkens [15, 16]). The situation in the circadian system of *A. mexicanus* seems to be comparable. Since back crosses of *A. mexicanus* have well functioning eyes (Wilkens [15]), it would be of interest to see whether the circadian system behaves in a similar way if the Pachon cave form is crossed with the epigean river form.

Maybe, there is another reason for the lasting existence of the circadian oscillator. Perhaps, the cave inhabiting bats with their clearcut day-night-rhythm could act as at least weak pacemakers. Nevertheless, there is no obvious advantage for the cave fish for keeping the circadian rhythm. The food supply in the Cueva de El Pachon is sufficient, even in times when bats are hunting outside the cave (Wilkens, pers. inform.). In general, this speculation seems to be less probable than to think of the circadian system as a relic.

One could object that the ontogenesis of the tested specimens under LD-conditions had an influence on the endogenous time control, and that specimens grown up in constant darkness will show a different behaviour. To study these problems, a further paper is in preparation.

Acknowledgements

The authors wish to thank Prof. Dr. J. Parzefall, Prof. Dr. F. Weber, Prof. Dr. H. Wilkens for comments on the manuscript. Moreover, the authors want to thank Prof. Dr. J. Parzefall and Prof. Dr. H. Wilkens, who placed the *Astyanax* specimens at their disposal. Experiments have been performed at Prof. F. Weber's laboratories in Münster i. W. as part of the research project "chronobiology of caverniculous animals".

This study was made possible by grants of the Deutsche Forschungsgemeinschaft (We 389 and Ma 895).

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