ノバリケン胚における心拍リズムの発達

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Development of Heart Rate Rhythmicity in Muscovy Duck Embryos

Joachim HÖCHEL*, Elmar MOHR*, Martin NICHELMANN**, Ralph PIROW***and Hiroshi TAZAWA****

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The heart rate (HR) of Muscovy duck embryos (*Cairina moschata f. domestica*) was continuously recorded from as early as the 21st day of incubation (D21) until hatching (D34/35). The aim of the study was to investigate the influence of phonoperiods consisting of different acoustic stimuli on the course of HR and the development of HR periodicities during this period. Incubation was carried out at a constant temperature and in constant darkness. Until D25 HR was dominated by decelerative fluctuations only, indicating a main input from the parasympathetic system on the heart. Later sympathetic influences increased progressively. HR periodicity was investigated by means of χ^2-periodogram and fast Fourier transformation. Between D26 and D30 statistically significant and stable HR periodicities developed gradually. They had periods in the range from 5 to 38 hours. Ultra-, circa- and infradian rhythms (< 20 h, 24 ± 4 h and > 28 h, respectively) occurred in parallel in some cases in the same embryo. During these important periods HR courses were dissimilar between individual embryos and had different intensities. There was no indication that acoustic stimulation (phonoperiods) had any effect on the development of HR periodicities.

Keywords: ultradian and circadian rhythms; muscovy duck; embryonic heart rate; heart rate irregularities; functional innervation of the heart; acoustic stimuli; phonoperiods; chi square periodogram; 1/f-characteristic

1 INTRODUCTION

The development of circadian rhythmicity has become a topic of outstanding interest during recent years. Although the identification of "clock genes" has been successfully carried out in some species, invertebrates and vertebrates, (e.g. [48]), we are still in search for physiologic mechanisms of rhythmicity development during ontogeny.

Because of their, in comparison to mammalian fetuses, convenient accessibility and because of their possible ontogenic development disconnected from rhythmic maternal influences, i.e. in an incubator, bird embryos seem to be an ideal object for this sort of rhythmicity research. For immediately after hatching locomotor activity of chicken chicks showed a clear circadian rhythm[4,7], it is sensible to search for a prenatal development of circadian rhythms. It is likely that these locomotor rhythms develop already prenatally, as developing circadian rhythms showed in general increasing amplitude[49,67].

It was reported in the past that there is a strong correlation between locomotor activity and heart rate[5,8,15,34] suggesting that heart rate (HR) might be a meaningful parameter for assessing the perinatal development of the avian circadian system. HR measurements have the advantage that they can be carried out noninvasively and continuously in the same embryo at least during the second half of incubation[2,35,44,47,55].

HR is directly controlled by the autonomic nervous system via the vagus and the sympathetic nerve and by circulating catecholamines. Hence, rhythmic HR changes are necessarily connected with functional cholinergic and adrenergic cardiac receptors, fast and short-term HR alterations also with a functional autonomic innervation of the heart. Previously reported HR irregularities (HRI) in chicken embryos[22] gave further proof that the embryonic chicken heart is functionally innervated by the vagus nerve around day 12/13 of incubation (D12/13), sympathetic influences were noted from D15 onwards. These HRI are sudden deviations of HR from its baseline values of more than 10 beats per minute (bpm) and were not only reported for the chicken embryo but also in other avian species like king quail[44], budgerigars and crows[42].

* Freie Universität Berlin, Germany
** Humboldt-Universität zu Berlin, Germany
*** Westfälische Wilhelms-Universität Münster, Germany
**** Department of Electrical and Electronic Engineering

The occurrence of HRI patterns as an indication of a functional cardiac innervation raised the question if in addition to these very fast and short irregular HR alterations periodic HR changes in the circadian range develop prenatally and which environmental factors they are dependent on. As shown by several authors[4,7,49] body functions in chicken are subjected to a circadian/diurnal rhythm immediately after hatching. In order to investigate the prenatal development of such periodicities an avian species with a long incubation period is particularly suitable. That is why this paper concentrates on results in the Muscovy duck, a precocial species with an incubation period of 35 days. A first step in these investigations and the aim of this paper was to determine if circadian HR rhythms develop prenatally in Muscovy ducks and if acoustic stimuli can act as a zeitgeber for a HR rhythm during embryonic development.

2 MATERIALS AND METHODS

Fertile eggs of Muscovy ducks (*Cairina moschata f. domestica*) were incubated at 37.5 °C and 60 % relative humidity. Incubation took place in constant darkness. Until the start of experiments eggs were turned continuously. The age of the embryos is given in days of incubation designating the first day as D1.

Embryonic HR measurement started between D21 and D25 and was carried out continuously until hatching, which occurred on D34 and D35. The method of HR recording is published elsewhere[46,47]. In brief, HR recording was based on detection of electrocardiograph signals (ECG). In order to place the ECG electrodes the eggshell was removed at three opposite sites 1 x 5 mm in size. Electrodes were stuck between eggshell and outer shell membrane. The electrocardiogenic signal was amplified and high- and low-pass filtered. Each R-peak of the ECG greater than an adjustable trigger threshold was detected via a threshold switcher by a connected computer. The frequency of this ECG device output monitoring was 500 Hz.

After 1 h of sampling the data were stored on a removable hard disk and recording started again. Hard- and software allowed the parallel recording of eight embryos. The sampled raw data, which were in fact inter-beat-intervals (in ms), were afterwards converted into an equidistant time series of HR data averaged over 1 s for investigating short-term alterations and over 30 s for searching for circadian rhythms.

HR during avian embryonic development shows not only short-term fluctuations like the HRI mentioned above[22], but also a long-term trend. It was disclosed in several avian species that during the second half of incubation HR drops until a minimum at the time of internal pipping (IP) and then increases again sharply. Before the HR decrease a plateau phase of HR was observed on some species[55]. The course of HR in Muscovy duck embryos fitted this general description (Fig. 1A). Hence it became necessary to exclude this long-term trend from the data before a circadian time series analysis could be carried out.

In order to define mathematically the trend to be leveled out, the whole file was split into two parts; the division point was the HR minimum at IP, which occurred around D31. Over hourly mean HR data until and after IP a polynomic regression was calculated, its grade was dependent on the regression coefficient (R^2). For determing the grade of the polynome to be used for data detrending, regression formulas were calculated until the R^2 value was at least 0.8. Afterwards a regression formula of the same grade was calculated for the 30-s-HR-data, again until and after IP. The HR data were detrended by subtracting the corresponding regression value from the HR value (Fig. 1B).

The resulting time series of detrended HR data (HR_d) were subjected to time series analysis by means of χ^2-periodogram and fast Fourier transformation (FFT). For the values of a χ^2-periodogram, the so called Q_p-values, follow a χ^2-distribution, it was possible to define a level of significance, i. e. to determine periods in the HR_d data, which were with an error of 0.001 statistically significant[32,51]. FFT was used to confirm the found periods in the HR_d data with a second, methodically independent procedure.

Targeting more detailed information than just a significant periodicity in the HR data file, the method of χ^2-periodogram was slightly modified, i.e. simple mathematical alterations were added. Its main aim was to make Q_p-values of different periods and embryos comparable to each other. This was achieved by defining a relative Q_p-value, which is the difference between the calculated Q_p-value and the significance theshold for the respective period, divided by this threshold value.

$$Q_{p_{rel}} = \frac{Q_p - Q_{signif.}}{Q_{signif.}}$$

Hence the relative Q_p-value indicates the ratio of Q_p and respective significance threshold; relative Q_p-value greater than zero are statistically significant. The advantage of this data presentation is that Q_p and Q_{p+n} even with n >> 1 could easily be compared with each other (Fig. 2). In order to test the influence of periodic acoustic stimulation on the embryonic course of HR different periods of high sound level were experimentally arranged. Their duration was half an hour (experiment #2), four (experiment #3) and eight hours (experiments #4 and #5) per day, their period was 24 hours in all four experiments. One additional experiment (experiment #1) took place without any experimental acoustic stimulation. Three embryos of each experiment were subjected to HR time series analysis.

During the phonoperiods maternal calls,

duckling calls, rectangular waves of 800 or 1000 Hz, white noise and music were played back. The sound level in the incubator during the acoustic stimulation was between 70 and 80 dB, the mean background noise of the incubator was 62.6 ± 1.3 dB.

3 RESULTS

All HR courses recorded showed a uniform appearance in terms of their long-term trend (Fig. 1). Until the onset of lung breathing after IP HR decreased to mean values of 200 ± 11 min^{-1}. Afterwards HR values increased to 310 ± 17 min^{-1} and dropped one to one and a half days before hatching again. In several cases some hours before hatching another sudden HR increase was observed.

Variation of hourly mean HR increased during embryonic development (Fig. 1). Until D25 only minor fluctuation around the long-term trend could be observed, from this incubation day onwards HR variations became more obvious, their amplitude increased further until hatching.

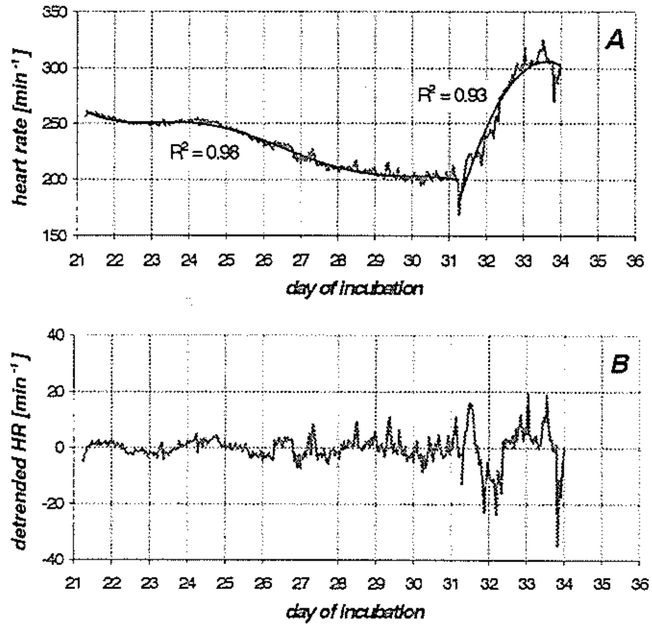

Fig. 1. A: Embryonic heart rate in a Muscovy duck. The grey line indicates hourly mean values, the black line the long-term trend calculated by regression formulas until and after the minimum of the heart rate course. The degree of the polynomic regression formula for the first part was 5, for the second part 2. The resulting R^2-values are noted. B: Detrended heart rate from Fig. 1A. The hourly mean heart rate values were subtracted from the long-term trend corresponding to the calculated regression formula.

If HR data was however analysed on a finer time scale (Fig. 3) short-term fluctuations of HR were visible from the beginning of HR recording, i. e. from D21 on. First a relatively constant baseline HR was still to be observed, from which downward deflections occurred. Later in incubation, i. e. around D25 deflections from mean HR occurred in both directions. Tachycardic events became dominant around D30.

As it is comprehensible from Fig. 1 and 3, just plotting the HR course as a function of time during embryonic development did not reveal signs of HR periodicity. In order to investigate if statistically significant periods were however hidden in the data the χ^2-periodogram was used.

First of all it was conspicuous that all periodograms showed in general increasing Q_p-values with an increasing test period. If the χ^2-periodogram was transformed into a double-logarithmic graph of Q_p versus test frequency f the well known $1/f$ characteristics could be seen (Fig. 4).

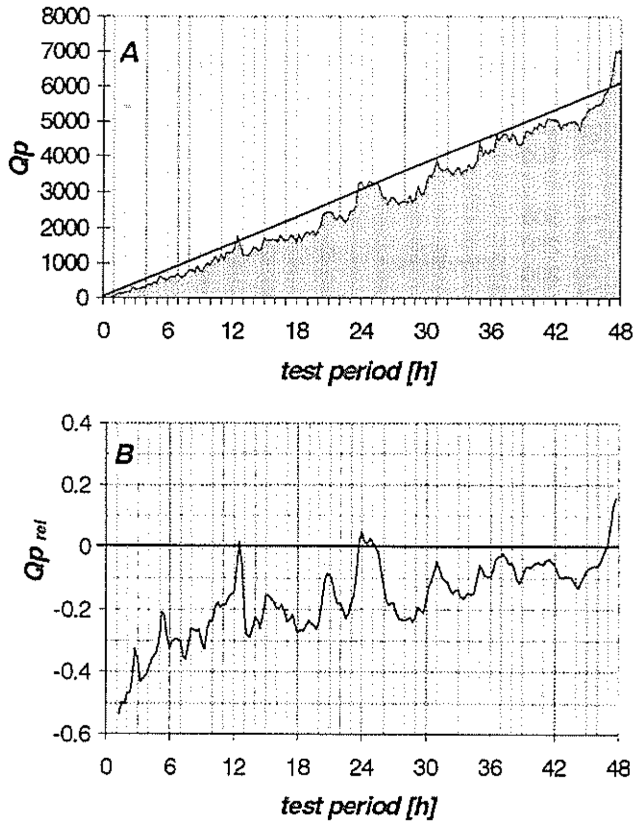

Fig. 2. A: χ^2-periodogram for the heart rate course shown in Fig. 1. The straight line indicates the level of significance (P = 0.001), i. e. Q_P-values exceeding this line are statistically significant. B: Modified χ^2-periodogram giving the relative Q_P-values corresponding to Fig. 2A. For details see text.

In addition to this general Q_p increase with increasing test period in a number of embryos certain periods had a significant (P = 0.001) Q_p-value, indicating that these periods were not random but a real property of the data analysed. Twelve of the 15 HR courses had statistically significant and stable periodicities in the ultradian (≤ 20 h), circadian (24 ± 4 h) and infradian (≥ 28 h) range (definitions following [11]). Three embryonic HR courses did not show any significant periodicity. There was however not any interrelation between significant periodicities in the HR data and the experimental acoustic setup. Neither did the embryos not experimentally sound exposed show any HR specifity. Although the sound level in the incubator had a clear 24 hour periodicity in all experiments only two out of the 15 embryos tested showed a significant 24 hour HR rhythm. Seven embryos had one or more ultradian HR periods ranging from 5 to 19 hours, circadian periods in the range from 20 to 28 hours

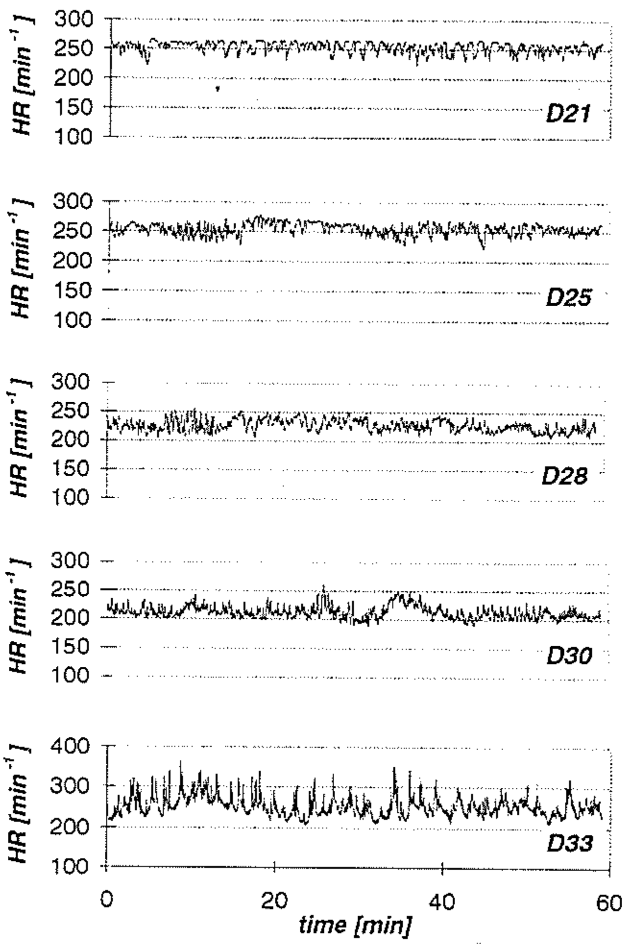

Fig. 3. Representative 60-minute parts of the course of heart rate on different days of incubation in the same embryo as presented in Fig. 1. Values were calculated from the inter-beat-intervals and transformed into an equidistant time series with a data point frequency of 1 per second. It becomes obvious that there was increasing amplitude of heart rate fluctuations with time of incubation. In addition to the firstly only occuring downward deflections of heart rate (D21), accelerative heart rate events develop gradually (D25-D30), until heart rate is dominated by accelerative heart rate patterns (D33). Beside brief heart rate alterations baseline fluctuations develop progressively. Please note the different scale of the Y-axis on D33.

were also seen in seven embryos, infradian periods from 29 to 38 hours in six embryos. Almost all embryos exhibiting any periodicity in their HR data had several significant periods (Fig. 5).

Beside the individually quite different values of significant periods in the HR data their intensity differed individually as well. As already mentioned above 3 of the 15 HR data files were aperiodic (Fig. 6). In four embryos a significant HR periodicity could be proven, there were

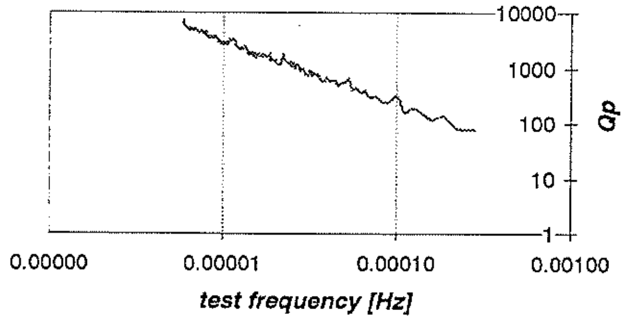

Fig. 4. Double-logarithmic representation of the χ^2-periodogram shown in Fig. 2. The in this form linear decrease of Q_P-values with increasing test frequency becomes obvious (1/*f*-pattern).

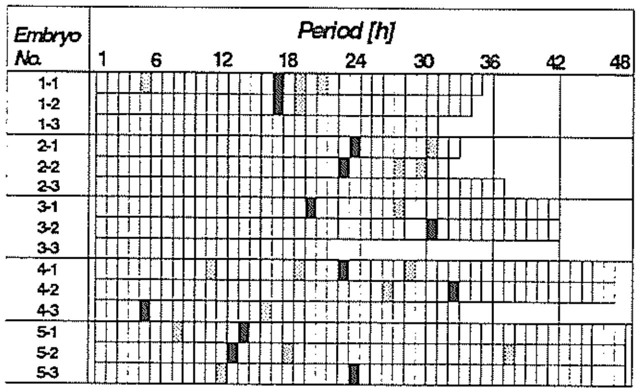

Fig. 5. Significant periods in the heart rate data of 15 embryos during the last ten days of incubation. Black boxes indicate the period showing the maximum relative Q_P-value for each embryo, grey boxes indicate additional significant periods. Embryonic heart rate was recorded in five experiments during which daily phonoperiods of half an hour (#2), four (#3) and eight hours (#4 and #5) were applied. Experiment #1 was the control and took place without any experimental acoustic stimulation.

however only minor differences between the Q_P-value of significant and non-significant periods. Hence, these HR data were categorized as low level periodicity. In addition, their relative Q_P-value was below 0.1. The next category defined was the medium level periodicity. Their characteristics were again a relative Q_P-value below 0.1, however significant periods stood out from the other test periods. Finally high level periodicity was denoted by markedly outstanding Q_P-values above 0.1, i. e. the Q_P-value was more than 10 % above the significance level. The distribution of HR data according to the so defined categories was balanced, for four embryos had to be assigned to each periodicity level.

This distribution of periodicity intensities together with the increasing HR fluctuations around the long-term trend (Fig. 1) suggested that there was a gradual development of HR periodicity during ontogenesis. This hypothesis was supported by the fact that Q_p-values of significant periods increased progressively when an analysing window was moved in 12 hour steps from the beginning towards the end of the data file (Fig. 7). None of the embryos showed significant HR periods before D26.5 and none of the embryos showing any HR periodicity before hatching did so later than D29.5. Mean day of incubation, at which the significance level for Q_p-values was passed, was D28 ± 1 day. Once the significance threshold was crossed the respective Q_p-values stayed stable above it. Although the method of χ^2-periodogram has in comparison to FFT the advantage of resulting in statistically testable period intensities, all results should be double-checked with another, mathematically independent test method. This demand seemed to be particularly reasonable, for in the original HR data periodicities could not obviously be detected (Fig. 1). All results obtained by χ^2-periodogram analysis were confirmed by FFT, i. e. periods with a significant Q_p-acme peaked also in the FFT power spectrum.

4 DISCUSSION

Embryonic HR is a sensitive indicator of physiological incubation conditions, for HR responses occur immediately after changes of incubation temperature[35,53,54,56], gas concentrations in the incubator[52], or lack of turning the eggs during incubation[43]. In our own experiments the course of embryonic Muscovy duck HR in terms of its long-term trend (Fig. 1) was in accordance with findings in other avian species[55]. Also for embryos of chicken, Japanese quail, Pekin duck and turkey a continuous fall of HR before IP followed by a sharp HR increase was described. Hence Muscovy duck embryos are appropriate to be taken as a model for other precocial avian species, despite their relatively long incubation, a fact which is advantageous for rhythm analysis. An equal pattern of HR in Muscovy duck embryos as found in our own experiments was described by Pirow[46] who carried out long-term recordings of HR from D25 until hatching. It can be concluded that the own incubation conditions used, including the experimental acoustic stimulation, did not have any abnormal impact on embryonic development.

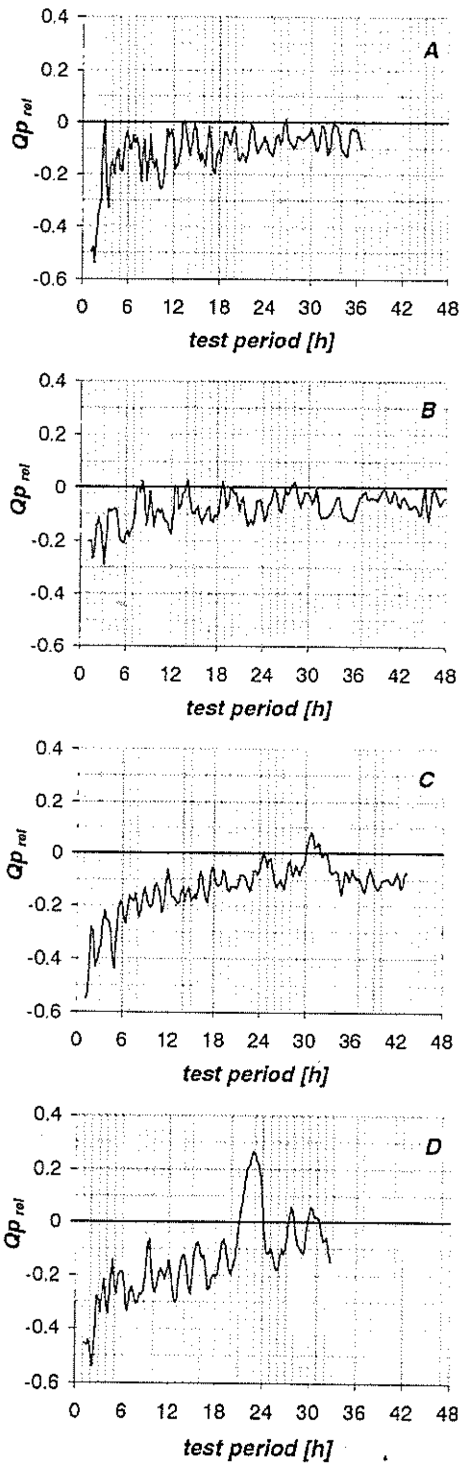

Fig. 6. Relative (modified) χ^2-periodogram of four embryos' heart rate. Different levels of periodicity intensity are obvious in addition to individually dissimilar significant periods in the heart rate course. Heart rate was classified as aperiodic (A), low (B), medium (C) and high level periodic (D). See text for details.

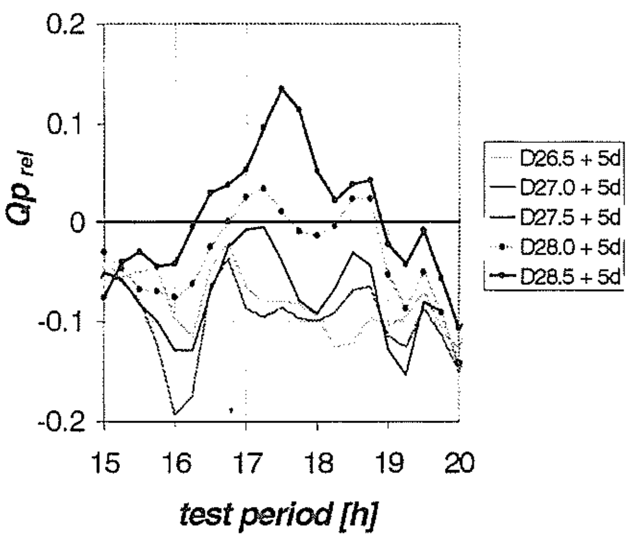

Fig. 7. Development of relative Q_P-values when a 5-day window of χ^2-periodogram analysis was moved through the data file. In this example Q_P-values become significant for the first time on D28, increase their intensity further on and stay stabile above the significance level.

Another characteristic of embryonic HR development are its short-term fluctuations (heart rate irregularities = HRI). They are an indication of a functional autonomic innervation of the heart. It was shown recently[22] that in chicken embryos decelerative HRI occur for the first time at the onset of an operative parasympathetic innervation of the heart, which is around D12[41]. These HRI disappeared after injection of atropine, a parasympathetic blocking agent, but not after administration of norepinephrine or isoproterenol, which are sympathomimetic substances. Accelerative HRI patterns were not affected by atropine. They occurred for the first time around D15, i. e. at the onset of functional sympathetic innervation of the heart[18,26]. If propranolol, a sympathetic blocker, was injected, the observed rapid accelerative HRI patterns disappeared, but not the decelerative HRI patterns[22].

Hence, from the occurrence of decelerative and accelerative HRI the onset of an operative autonomic innervation of the heart can be concluded. At the beginning of our HR recording in Muscovy duck embryos only decelerative HR patterns were observed. This indicated that at D21 parasympathetic innervation was functional, but that there was no effective sympathetic influence. The latter was seen for the first time around D25 as indicated by the appearance of accelerative HR patterns in addition to the decelerative ones (Fig. 3).

When HR is controlled via the autonomic nervous system it is plausible to search for inherent rhythmicities in order to unveal possible influencing mechanisms. One characteristic of all χ^2-periodograms was the found $1/f$-pattern. That means that Q_P-values increased linearly with increasing test period, i. e. the lower the frequency of the oscillation tested the higher was its intensity (Fig. 4). This $1/f$-characteristic is well known for many processes in nature, beside others also for human HR[25,27,31]. The cause of this phenomenon has not been known. It was suggested that it was generated due to the interaction of several control mechanisms working in different time scales[25]. For instance, blood pressure is regulated by a number of mechanisms, which operate and have an effect within seconds (baro- and chemoreceptors), within minutes (renin-angiotensin-system) or within several hours (renal fluid volume)[16]. It was speculated that biological systems, which show the described $1/f$-characteristic, work very stabile, for the can respond effectively on several time scales to environmental changes[30,31].

One of the environmental periodicities, which are of outstanding importance for all biological systems are the daily changes of light and dark (LD). They result in diurnal and circadian rhythms of organ functions (33). Although also for HR a diurnal and circadian rhythm was described in chicken[36,49], HR in general is a relatively frail chronobiological variable, for it is an effector part in several regulation, control and behavioural mechanisms, e.g. blood pressure, thermoregulation, respiration, excitement, locomotor activity. Hence, an existing HR periodicity could be masked by continual acute HR responses to exogenous stimuli. If circadian HR rhythms were found they would be indicative for a functional circadian system; however the non-existence of a circadian HR pattern does not at all rule out the latter.

There are at least two conceivable coupling mechanisms between the circadian system and HR. Firstly, the sympathetic output pathway of the suprachiasmatic nucleus (SCN) might influence the HR via the central nervous system. Secondly, pineal or retinal melatonin might modulate HR directly via cardiac melatonin receptors[37,39] or indirectly via melatonin receptors in the brain[38,40]. There are several reasons to propose that melatonin is involved in a coupling mechanism between the internal clock and HR in birds, which might be of importance already prenatally. One argument is the existence of cardiac and brain melatonin receptors mentioned above, another the existence of clear LD differences of pineal melatonin in chicken embryos not later than D16/D17[1,28], and finally there were acute HR responses to exogenous melatonin in chicken and Muscovy duck embryos commencing around D17 in chicken and D24 in the duck[21]. In the Muscovy duck all embryos tested responded to melatonin on D27[19].

These melatonin-related characteristics should facilitate a prenatal, melatonin mediated circadian HR rhythm. However, HR periodicities in the circadian range were found only in part in our own experiments. It should be noted though that there was a development of HR periodicities during prenatal ontogeny. When the start of periodic changes of the pineal melatonin contents is projected from chicken embryos (21 days incubation, start of LD changes in pineal melatonin not later than D16/17 [1,28]) to Muscovy duck embryos (35 days incubation) its pineal melatonin should commence fluctuating around D26 to D28. This calculation is in surprising accordance with the first appearance of significant Q_p-values in our HR analysis, which was between D26.5 and D29.5.

Natural rhythms develop usually with an increasing amplitude. This was proven for instance for pineal melatonin secretion in chicken embryos[28,66,67], the amplitudes of HR and locomotor activity in chicken chicks during the first week of life[4,49], or the amplitude of the body temperature oscillation in infants[64]. This general gradual increase of oscillation amplitudes would explain that in our own experiments different levels of periodicity intensities were found. It can be supposed that the HR course was recorded in a window of periodicity development, which did not take place synchronously in all embryos. Although all but three embryos showed some degree of HR periodicity, some of these seemed to be already further advanced in terms of fluctuation intensity, others a little behind.

The results raise however one main question: Why do the HR periods differ so much from 24 hours, although there was a 24-hour-periodic environmental change, the experimental sound exposition. Four possible causes shall be considered here:

Firstly, might it be possible that there was no perception of the acoustic stimuli? It was shown previously[20] that in Muscovy ducks from D27 onwards the same acoustic stimuli as used in the experiments described here, caused immediate HR responses. These HR responses were a rapid rise or fall in HR or an increase of beat-to-beat variability. These responses gave proof that not later than D27 the afferent pathway from the ears to the central nervous system, the autonomic innervation of the heart and the coupling of both were functional. In addition, the acoustic stimuli used must have been of some physiological or behavioural importance for the embryo, for it caused an activation of the autonomic nervous system.

Secondly, is the HR a useful indicator of a functioning circadian system? As mentioned above HR is part of several physiological control and regulatory systems. If these body functions are out of phase to each other a HR periodicity might be levelled out. Although in many experiments a strong correlation between different body functions, e. g. locomotor activity, metabolism, and HR was shown [8,15,34,48,57], in constant light there was not a circadian rhythm of chicken HR during the first week of life[49] despite a circadian rhythm of locomotor activity[4,7]. In LD however a diurnal HR rhythm was shown and persisted in constant darkness[49]. In conclusion it has to be noted that HR is at least a very variable parameter from the chronobiological point of view.

These results link up to the third consideration: Is periodic acoustic stimulation at least a potential zeitgeber for the circadian system? It is out of question that light is a much stronger zeitgeber than sound, and there are some morphological explanations for that. One is that in several birds one circadian oscillator is situated in the retina. In addition, the avian pineal gland is photoreceptive. And there is via the retino-hypothalamic tract a direct connection of the eyes to the third avian circadian oscillator, the SCN. However, in adult chicken and quail also phonoperiods (instead of photoperiods) of 12 hours appearing every 24 hours were effective zeitgebers for a circadian rhythm of body temperature and locomotor activity[9,10]. It is also well-known that acoustic stimuli have an important impact on embryonic development during late incubation in several bird species as was shown in many experiments using click sounds for hatching synchronization[58,59,60,61,62,65]. Hatching synchronization by click sounds was also observed in the Muscovy duck[29]. The results presented in this paper suggested however that acoustic stimulation, although successfully percepted as indicated by acute HR responses, had no zeitgeber effect on HR rhythmicity.

A fourth consideration concerning the lack of phase locking between phonoperiod and HR period targets the responsiveness of a developing endogenous HR rhythm to a potential zeitgeber. It might be speculated that an endogenously developing HR periodicity was hit by the tested acoustic zeitgeber in an uneffective phase[3,5], so that an entrainment could not occur. Unstimulated embryos showed however for instance a 17-hour HR period (Fig. 5). If we consider this as a free-running endogenous rhythm all its phases would coincide with parts of a daily 8-hour phonoperiod at least once within four days. If the zeitgeber tested were effective, a phase response should be seen at some stage. A phase advance or delay would cause a decrease of Q_p[19]. Our results showed however a continuous Q_p-rise in consecutive 12-hour intervals (Fig. 7).

We attribute the lacking entrainment of the HR rhythm by the exogenous acoustic stimulation mainly to the morphologically and functionally loose connection of the acoustic input and the internal clock, and to the fact that HR rhythmicity was just appearing during our window of recording. Hence it might be of future interest to continue the experiment after hatching. This would also give way to a longer time of possible entrainment. The fact that 24-hour periodic phonoperiods did not cause a dominant 24-hour HR rhythm has important implications for the design of further experiments concerning the effect of zeitgebers on the establishment of HR periodicities during embryonic development, for it suggests that in such experiments a totally sound proof environment is not necessary. The span of mean sound level from 59 dB to 80 dB, which was applied in our own experiments and which had no zeitgeber effect on HR periodicity, is a span what is well above usually in physiological laboratories occuring sound levels.

The appearance of several significant peaks in the spectrum of the χ^2-periodograms (Fig. 5) is a finding, which is in accordance with results in other young animals. In chicken chicks beside dominant 24-hour periods of locomotor activity other significant ultradian periods were found in the same animal[7]. Similar results were obtained in newborn mammals[12,13,50]. It was supposed that ultradian rhythms are like circadian rhythms inherited. A special importance was attributed to ultradian rhythms during periods of particular pressure or transition of physiological functions of the organism, when the intensity of circadian rhythms decrease and the power of ultradian rhythms increase. This was found for instance in mice during the weaning period[63]. The last trimester of incubation in birds is definitely such a phase of transition.

In addition it was postulated that the formation of diurnal/circadian rhythms depends primarily on the maturity of the particular organ or regulatory loop investigated. Generally, the early postnatal period is characterized by the dominance of ultradian rhythms, only during further ontogeny their power decreases in favour of circadian rhythms[14,17,23,24,63,64].

In summary, the ultra- and circadian HR periods found in Muscovy duck embryos during the pre- and perinatal period can be interpreted as a reflection of developing periodicities of organ functions. The dominance of periods < 24 hours was in accordance with findings in human infants and mammals, that first in ontogeny ultradian rhythms govern the organism before the circadian rhythms become dominant. For in some embryos HR periods in the circadian range were already presiding, the perinatal period seems to be of outstanding importance for their development, so that they are manifest after hatching. Periodic acoustic stimulation does not seem to have an impact on the development of HR periodicity.

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ノバリケン胚における心拍リズムの発達

ヘーヒェル ヨアヒム*、モール エルマー*、ニッケルマン マーチン**、ピロウ ラルフ***、日澤 皓****

概要

ノバリケン（タイワンアヒルの原種）の胚心拍数を孵卵 21 日目から孵化する 34-35 日目まで、心拍数成長パターンと心拍周期性の発達に及ぼす音刺激期間の影響を明らかにするため連続測定した。孵卵は一定温度と暗闇の中で行った。孵卵 25 日目まで、心拍数は徐脈性ゆらぎのみ現れ、副交感神経の作用を示唆した。その後次第に交感神経作用が増してきた。心拍周期性は χ^2-ペリオドグラム法と高速フーリエ変換により検証した。孵卵 26 日から 30 日目の間、有意で安定した心拍周期性が次第に発達した。それらの周期は 5 時間から 38 時間に及び、ウルトラディアン（20 時間以内の周期）、サーカディアン（24 ± 4 時間の周期）及びインフラディアン（28 時間以上の周期）リズムが同一の胚において、いくつかのケースについて並行して現れた。これらの期間、心拍数成長パターンは個体によって異なり、その大きさも異なった。心拍周期性に対する音刺激の効果は示唆されなかった。

キーワード：ウルトラディアンとサーカディアンリズム、ノバリケン、胚心拍数、心拍不規則性、心臓神経支配、音刺激、音刺激期間、χ^2-ペリオドグラム、1/f 特性

*ベルリン自由大学、**フンボルト大学、***ヴェストファーレン大学、****電気電子工学科