



## 成長するニワトリ雛における心拍リズム

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# Cardiac Rhythms in Developing Chicks

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Chick instantaneous heart rate (IHR) was determined by electrocardiogram measured noninvasively from day of hatch to day 6. In experiment I, IHR was measured for 1-h periods twice a day, in daytime and at night, to investigate heart rate fluctuations (variability and irregularities). Chick IHR was substantially arrhythmic and spontaneous tachycardia of 100-150 bpm dominated HR fluctuations. We categorized the fluctuations into three types; 1) type I as a widespread baseline HR (20 to 50 bpm) due to respiratory arrhythmia, with a mean oscillatory frequency of 0.74 Hz (range 0.4-1.2 Hz); 2) type II as low frequency oscillations in HR baseline, at a mean of 0.07 Hz (range 0.04-0.10 Hz), of uncertain origin; and 3) type III as non-cyclic irregularities, dominated by frequent transient tachycardia. In experiment II, we determined the diurnal rhythms of chick IHR over 24-h periods and under conditions of a natural photoperiod, thermoneutrality and with feed available throughout the first week after hatching. Chick IHR was very variable in the daytime (250-500 bpm), due in part to feeding and activity, and decreased to a diurnal low (200-350 bpm) at night, when mean HR was stable. HR fluctuations persisted throughout the diurnal cycle.

Keywords: chick, diurnal rhythm, electrocardiogram, heart rate fluctuations, heart rate irregularities, heart rate variability, instantaneous heart rate, spectrum analysis

## 1 INTRODUCTION

In birds, embryos develop within the confines of hard eggshell and hatch after certain period of incubation. The heart is formed from a single tube to a four-chamber configuration during the early period of incubation. In chick embryos which hatch after about 21 days of incubation, the primordial heart begins to beat at around 30 hours of incubation<sup>(17)</sup> and the heart rate (HR) increases asymptotically during the early period to a level of about 280 beats/min (bpm) on average on days 16-18 with a subsequent decrease prior to pipping the chorioallantoic membrane (referred to as internal pipping, IP) and the eggshell (external pipping, EP) (see <sup>26</sup>). The embryos begin to breath air in the air cell with IP and from the surrounding atmosphere during the EP period and embryonic HR increases again more than 300 bpm<sup>(25)</sup>. After hatching, the HR decreases temporarily to about the level of pre-pipping values and then tends to increase during the first week of posthatching life<sup>(19,29)</sup>. These daily changes in HR during development (i.e., developmental

patterns of HR) were elucidated by measurements of mean heart rate (MHR) over short periods every day. We also have determined the developmental patterns of MHR in various species of precocial and altricial birds for comparative studies<sup>(1,2,8,12-15,22,23,25,27,30,31)</sup>. In these measurements, it was suggested that HR became variable toward the end of incubation and HR changes seemed to be augmented further after hatching. Based on these suggestions, instantaneous heart rate (IHR), which is calculated from the beat-to-beat intervals of the heart, was measured and the development of heart rate fluctuations (variability and irregularities) was investigated in chick embryos aged from day 12 to day 20 prior to IP (referred to as the prenatal period and the prenatal embryo), by both the acute, invasive catheterization method<sup>(11)</sup> and the noninvasive, continuous method of acoustocardiography<sup>(1,3)</sup>. In addition, IHR during IP and EP period (referred to as the perinatal period) was measured by electrocardiography and the development of HR fluctuations of internally and externally pipped embryos (perinatal embryos) was also investigated<sup>(28)</sup>. The HR baseline was flat until about day 13-14 of incubation and then the first episodes of transient, rapid decelerations and accelerations of HR (i.e., HR irregularities)

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occurred on around day 14 and day 16 of incubation, respectively, and HR variability and irregularities during the prenatal period were augmented toward IP<sup>(11,28)</sup>. HR fluctuations were further enhanced during the perinatal period toward hatching, and development of particular patterns of HR fluctuations was found during EP when the embryo became active in order to escape from the eggshell<sup>(28)</sup>. Hatching provides the embryo (which becomes a chick) a wide space for movements and locomotion and altered environments. Past experiments showed significant changes in chick MHR during the first week of posthatching development, suggesting that significant short-term changes in the HR of individual chicks occur and HR changes over the diurnal phase<sup>(19,29)</sup>. The present study was designed to provide IHR data in newly hatched chicks in order to continue our investigation of HR fluctuations from that of prenatal and perinatal embryos and ascertain diurnal rhythms of HR.

## 2 MATERIALS AND METHODS

### 2.1 Experimental set up

Broiler chicken eggs were brought from a local hatchery which also provided us with eggs for previous measurements of embryonic IHR<sup>(1,11,28)</sup>. Eggs were incubated at 38 °C and about 60% relative humidity in a forced-draught incubator and turned automatically every hour for normal embryonic development<sup>(23)</sup>. On day 17 of incubation, eggs were moved to a still-air incubator for hatching. After chicks hatched, they were transferred to a heated brooder, and supplied with commercial poultry feed and water *ad libitum*. Individual chicks were identified by uniquely coloring their down. Flexible Ag/AgCl gel electrodes 2 cm diameter and 2 mm thick (Vitrode A-50, Nihon-Kohden, Tokyo) were used for measurement of electrocardiogram (ECG). Three electrodes were attached to the skin by the adhesive gel at the lateral thoracic wall under both wings, and at the ventral abdomen, caudal to the sternum. Electrode wires were fixed above the chick's back with adhesive tape so that it could move freely within a measuring cage 25x20 cm wide and 20 cm high. During ECG measurement, the chick was confined in the measuring cage with feed and water and the cage was placed in a still-air incubator at 35 °C. HR measurement on the day of hatching (referred to as day 0) was made within 12-hour of emergence from the egg.

### 2.2 Experiment I

Experiment I was made to investigate the patterns and development of heart rate variability and irregularities and their relationships to the phase of day. ECG was measured for a 1-hour period twice a day (in daytime and at night) in 10 chickens from day 0 to day 6. Measurements were started 1 hour after placing the chick in the measuring cage

inside the incubator to allow for thermal acclimation and the chick to become quiescent. Daytime measurements were made under light conditions using a fluorescent lamp (08:00 - 17:00), and night measurements were made under dark conditions (18:00 - 04:00).

### 2.3 Experiment II

This experiment was designed to investigate the development of circadian rhythms in HR. Measurements were made using the same experimental set up as Experiment I except light conditions. A chick was fitted with the electrodes and ECG was recorded continuously for 24-hour periods, from day 0 to day 6, interrupted by a 1-hour intermission at the end of each of 24-hour measurement to save the data of each computer file onto a hard disk. During the intermission, the chick was weighed for body mass and given additional feed and water and the electrode positions were adjusted as required. No supplementary light was provided in the still air incubator, but dim natural light entered the incubator through windows in the door. A single chick was measured at one time from day 0 to day 6. The experiment was repeated over a period of 2 months, until 5 chicks were measured.

### 2.4 Calculation of IHR, MHR and power spectrum

ECG signals from the electrodes were amplified by 40-60 dB and bandpass-filtered with a cut-off frequency between 30 and 300 Hz. Amplification and cut-off frequencies depended upon the magnitude of the ECG signal and noise levels. Calculation of IHR with an error in accuracy of less than 1 beat/min (bpm) requires recording of ECG waves on a computer at a sampling frequency of no less than 4,000 Hz. Because it is not possible to save on the computer the whole ECG signals sampled at such a high frequency for even 1 hour, we recorded time intervals between the adjacent R waves on the computer using a program based on a threshold method and determined IHR continuously for 1 or 24-hour periods. The amplified ECG signals were sampled by an analog-to-digital converter at 4,000 Hz and the sampled signals were compared against a threshold level set on the computer. The threshold level was set above background noise levels to detect the raising deflection of sampled R waves. The times at which the magnitude of sampled R waves exceeded the threshold for the first time were stored successively in the data file of the computer. IHR was then calculated from the time interval between adjacent R waves.

In order to determine whether the HR fluctuations were cyclic (or oscillated) and the oscillation frequency in the case that they oscillated, power spectrum analysis of IHR recorded for 10-min period was made by a fast Fourier transform (FFT). Because the time intervals between individual IHR's were not equal, IHR data given by a series of points with

equal time interval (i.e., IHR per beat) were divided every 512 points (whose time series duration was dependent upon the HR; e.g., about 1.5 min for HR of 250 bpm) first from the beginning of 10-min recording and then from the first 257 points of the same 10-min recording. IHR data comprising each series of 512 points were then calculated by FFT for power spectrum. Because the abscissa of the power spectrum was expressed as 'per beat', it was converted to the frequency using the mean value of 512 IHR's of each section. The spectra of about 13-17 sections were averaged in order to improve resolution of the spectral peak.

IHR data determined in Experiment II were averaged every 5-sec interval or 1-min interval for mean HR ( $MHR_{5s}$  and  $MHR_{1m}$ , respectively) in order to demonstrate 24-hour or 7-day recordings of HR in a single graph.

### 2.5 Visual inspection of ventilatory movements

In some recordings of IHR, baseline HR seemed to oscillate with magnitude of 20-50 bpm and subsequent power spectrum analysis indicated the oscillation frequency of about 0.7 Hz on average, suggesting that these HR oscillations might be respiratory arrhythmia. Additional experiments were made as a supplement to Experiment I, in which the status and ventilatory movements of the chicks were noted with time during 1-hour measurements of IHR. The experiments were made outside the incubator at a temperature warmed by an electric lamp put in the measuring cage. Room temperature ranged between 20 and 25°C. The HR fluctuations, particularly baseline HR oscillation, were examined with the status and ventilation frequencies of chicks noted visually.

## 3 RESULTS

### 3.1 Experiment I

Figure 1 shows examples of 1-hour measurements of IHR in chicks aged from day 0 to day 6. Individual values of IHR are presented by single points. The points scattering above and below the HR baseline were artifacts caused by noises that exceeded the threshold. The noises of large magnitude occurred and intervened between successive peaks of ECG, particularly when chicks moved. The HR baseline was generally between 200-300 bpm in early days with a trend of higher HR toward the end of the week. In addition, the HR baseline recorded during 1-hour periods was higher in daytime than at night in most of the chicks, and in some chicks the baseline HR changed more than 100 bpm within 1-hour periods (e.g., last 2 panels in Fig. 1). While the HR baseline was widened occasionally up to about 50 bpm when the HR was lowered below 300 bpm, spontaneous accelerations in IHR

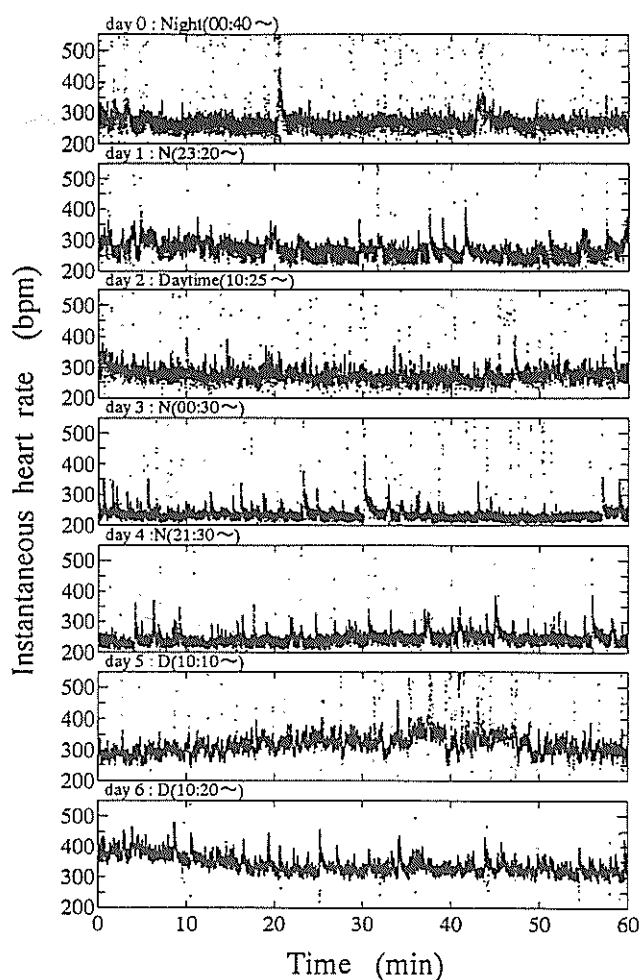


Fig. 1. Examples of 1-hour recordings of instantaneous heart rate (IHR in bpm) in chickens from day 0 to day 6 of posthatching. 'D' indicates examples of measurements made during the daytime, and 'N' at night. The numerical figures in the parentheses indicate the starting time of 60-min measurements.

exceeding 100-150 bpm and reaching 400-500 bpm occurred intermittently during 1-hour periods, independent of the phase of day, throughout the first week. These intermittent accelerations in IHR were rapid and brief, i.e., HR reached a peak in a few seconds and returned to the baseline within 30 sec.

Chick IHR was substantially arrhythmic and dominated by transient accelerations. These HR fluctuations were categorized into three types based on the patterns and periodicity of changes. Figure 2 shows three types of HR fluctuations presented in 20-min recordings. Type I had a wide spread baseline, which extended about 20 to 50 bpm (top panel). Type II was oscillatory changes in IHR having variable frequencies (II-A and II-B as shown in the 2nd and 3rd panels). Type III was characterized by non-cyclic irregularities (bottom panel).

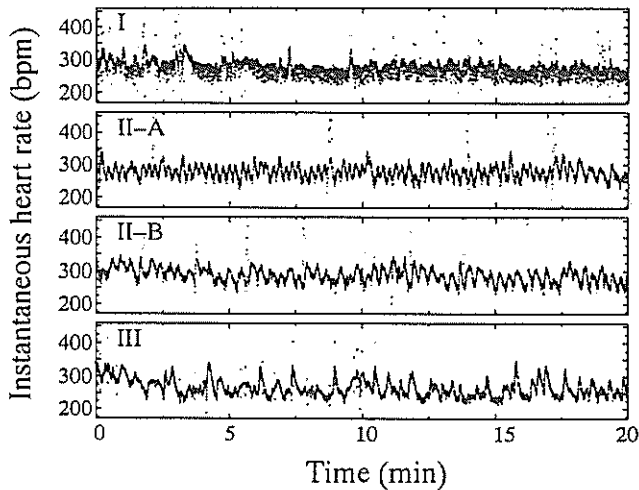


Fig. 2. Examples of the instantaneous heart rate fluctuations categorized to Type I, II and III, respectively, based on patterns of heart rate change and their periodicity. Type II patterns varied in their oscillation frequency (panels II-A and II-B). Chicks were 0-day-old for top panel, 0-day-old for the 2nd and 3rd panels and 5-day-old for bottom panel recording, respectively.

Time expansion of Type I fluctuations shows that the widespread baseline was attributed to rapid oscillations in IHR as shown in Figure 3A. The baseline HR oscillated between 220 bpm and 260 bpm with a period of about 1 sec (lower panel in Fig. 3A). Figure 3B shows another example of Type I HR fluctuations combined with Type II fluctuations. Widespread changes in HR (200-260 bpm) were Type I fluctuations as indicated in lower panel of Figure 3B. The lower panel of Figure 3B also indicates that as IHR increased to a peak of 275 bpm, Type I fluctuations were diminished. Superimposed over the Type I fluctuations, Type II cyclic fluctuations occurred about once every 20 sec throughout the recording (upper panel in Fig. 3B). In general, the three types of HR fluctuations often occurred independently or in combination during the 1-hour recording periods in all chicks, both in daytime and at night, as illustrated in an example from a day 0 chick (Fig. 4). In Figure 4, the first 16 min showed Type III irregular fluctuations, then Type I fluctuations joined Type III for about 8 min and from 24 min Type I fluctuations dominated, accompanying occasional tachycardia, until Type I oscillatory fluctuations were combined with Type II cyclic fluctuations at about 51 min. MHR with standard deviation during these four periods was  $306 \pm 26$  bpm ( $N=4785$ ),  $289 \pm 32$  bpm ( $N=2350$ ),  $255 \pm 32$  bpm ( $N=6652$ ) and  $259 \pm 24$  bpm ( $N=2183$ ), respectively.

In order to determine the frequency of Type I oscillatory fluctuations, Type II cyclic fluctuations and combined fluctuations of

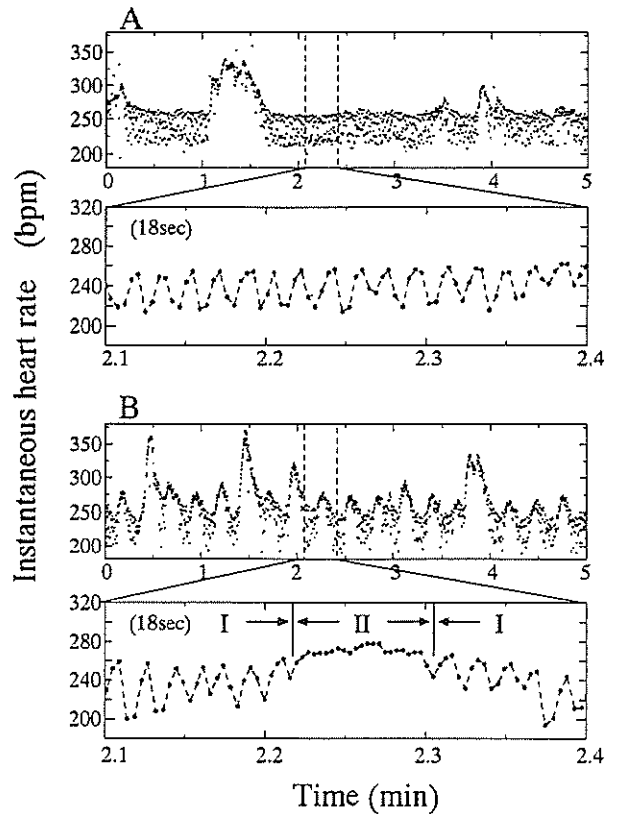


Fig. 3. Time expansion of Type I heart rate fluctuations. Panel A: an example showing that the widespread baseline (about 50 bpm in upper panel) is attributed to rapid oscillations in instantaneous heart rate as presented during 18-sec recording in lower panel. Panel B: another example of Type I heart rate fluctuations combined with Type II fluctuations. The bottom panel shows 18-sec recording extracted from the upper panel indicated by two broken lines and 'II' indicates a single peak of Type II fluctuations.

Types I and II, and Type III irregular fluctuations, IHR over 10 min periods was analyzed for power spectrum (PS) (Fig. 5). Panel A shows Type I fluctuations and the PS determined from a day 0 chick. A spectral peak was evident at about 1 Hz, as indicated by an arrow and symbol I. Panels B and C show Type II cyclic fluctuations with different frequencies determined from another 0-day chick and their PS. The frequencies indicated by spectral peaks were 0.073 Hz (panel B) and 0.047 Hz (panel C), respectively. Panel D shows Type III irregular fluctuations determined from a 5-day chick, which comprized intermittent accelerations of HR and showed no definitive peaks in the PS, indicating that HR fluctuations were irregular. Panel E presents a combination of Type I and Type II and the PS having two peaks at 0.96 Hz (I) and 0.066 Hz (II). In all the chicks measured for IHR during 1-hour periods in daytime and at night, Type I oscillations lasting for 10-min periods were observed in 51 cases and the oscillation

frequency averaged  $0.742 \pm 0.229$  (SD) Hz over a mean HR range of 220–300 bpm, and did not differ significantly between daytime and night (two-tailed t-test;  $t = 0.254$  n.s.).

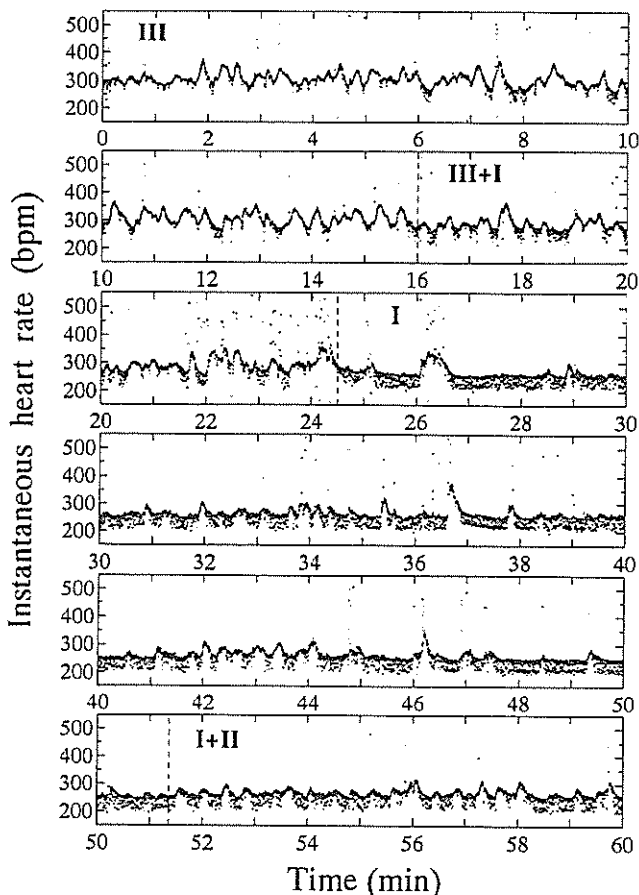


Fig. 4. An example of a 1-hour recording showing some of the three types of heart rate fluctuations, which often occurred in combination. A chick was 0-day-old and measurement was made at night.

The frequency of Type II cyclic fluctuations was on average  $0.069 \pm 0.015$  (SD) Hz ( $N = 27$ ) and did not differ significantly between daytime and night (two-tailed t-test;  $t = 0.0918$  n.s.) over a MHR range of 250–440 bpm, although MHR was lower at night.

In additional experiments, visual counts of ventilatory movements of the chest wall (while standing) or the humeral-scapular joint and wings (while sitting) indicated that the ventilation frequency ranged from approximately 30 times/min to 90 times/min while chicks were sleeping, calling and walking. IHR data recorded during 1-hour visual observations were examined together with the status of the chicks and ventilation frequency. The ventilation frequency was relatively high during calling and walking compared with that during sleeping; e.g., it was about 70 times/min on average during calling and walking and decreased to not more than 40 times/min when a chick slept during the last half of a 1-hour recording. The calling frequency was generally higher than the

ventilation frequency; it exceeded 100 times/min while chicks were sleeping, IHR was relatively low and HR baseline broadened, showing Type I fluctuations. The ventilation frequency counted visually was almost consistent with the frequency corresponding to the spectral peak; e.g., a chick breathed 30 times during a 7-min sleeping period (0.716 times/sec) and the spectral peak analyzed for IHR data during the same period was recorded at 0.722 Hz. In many cases, Type I fluctuations changed to Type III with augmented, irregular accelerations of HR when sleeping chicks were disturbed and awoke.

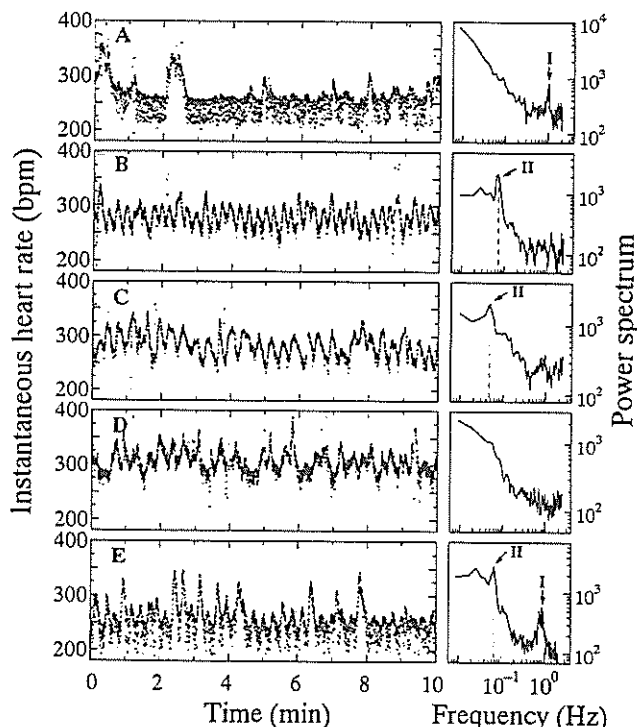


Fig. 5. Three types of heart rate fluctuations (panels A-D) and a combination pattern (panel E) during 10-min recording used for power spectrum analysis and their resultant power spectra. Dotted lines and arrows with symbols (I and II) in the right panels indicate spectral peaks. Chicks were 0-day-old for panel A, 0-day-old for panels B and C, 5-day-old for panel D and 1-day-old for panel E, respectively.

### 3.2 Experiment II

IHR was recorded continuously for 24-hour periods over the first week after hatching in the same chicks ( $N = 5$ ). IHR was averaged every 5 sec ( $MHR_{5s}$ ) in order to present time series patterns of HR changes during 24-hour periods in a single graph. Figure 6 presents examples of the  $MHR_{5s}$  of 5 individual chicks over 24-hour periods during the first week of posthatching. Measurements were started in the afternoon. As it became dark, HR decreased to a daily minimum. Baseline HR was disturbed by HR fluctuations in both daytime and night

phases of the 24-hour periods, but the baseline was most variable during the daytime. Baseline HR during the night was 200–250 bpm on the first few days and increased to 300–350 bpm on days 4–6. In addition, MHR scope (difference between day MHR

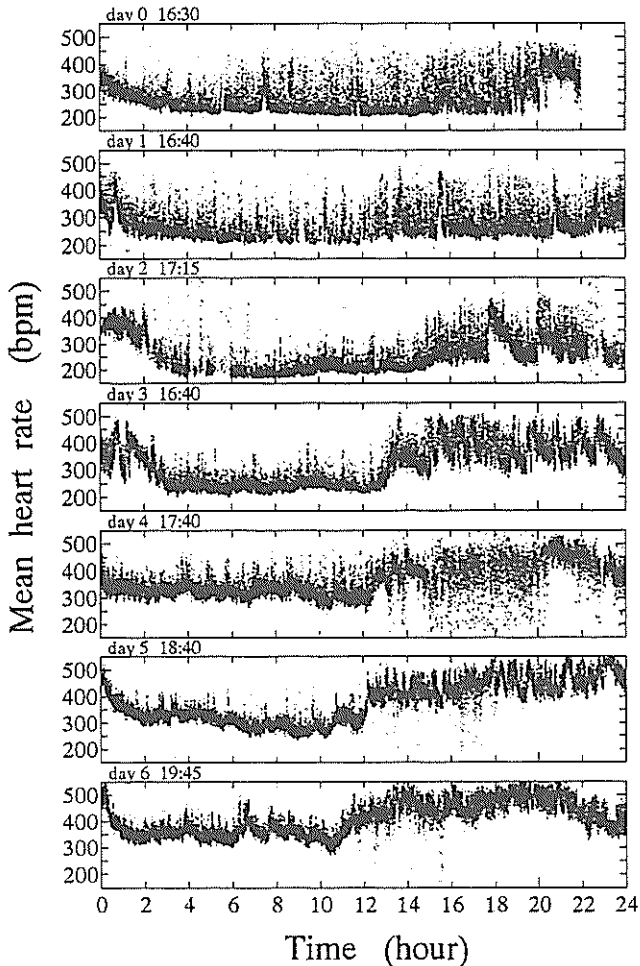


Fig. 6. Examples of mean heart rate every 5 sec ( $MHR_{5s}$ ) over 24-hour periods during the first week of posthatching in 5 chicks. Chick age and starting time of measurement are indicated above each  $MHR_{5s}$  recording.

and night MHR) increased with days toward the end of the first week, suggesting a diurnal rhythm of HR existed. Figure 7 presents an example of development of diurnal cardiac rhythms in a newly hatched chick. HR was averaged every 1 min ( $MHR_{1m}$ ) and individual points indicate  $MHR_{1m}$  to present development of HR changes during 1 week in a single graph. The MHR scope was about 50 bpm on day 1 and increased with development of the chick, showing clearly circadian rhythm of HR. Three other chicks also had clear circadian rhythm in HR already on day 1, but one remaining chick lacked a circadian rhythm until day 3.

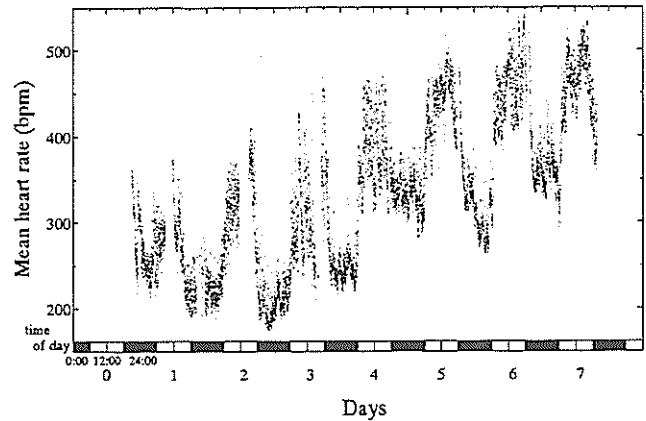


Fig. 7. Mean heart rate every 1-min period ( $MHR_{1m}$ ) plotted against posthatching age (days) in a chick. The horizontal axis indicates the time of day (hours), and black box indicates periods of darkness (night) from 6 pm to 6 am.

#### 4 DISCUSSION

##### 4.1 Patterns of heart rate fluctuations

The occurrence of fluctuations in the beat-to-beat interval of the heart rate (HR) in the developing human fetus and infant is well documented and several patterns of HR fluctuations are reported<sup>(6,7,10,16,20,32)</sup>. In birds, the development of HR fluctuations was recently investigated in prenatal and perinatal chick embryos<sup>(1,3,11,28)</sup>. These HR fluctuations were defined as either heart rate variability (HRV) or heart rate irregularities (HRI) depending on the magnitude of changes and their periodicity (cyclic or non-cyclic)<sup>(11,28)</sup>. In general, HRV is defined as oscillations in baseline HR, which often appear to be periodic, while HRI are larger fluctuations, comprising irregular, spontaneous decelerations and accelerations of HR. In newly hatched chicks, it was also found that the HR fluctuations occurred throughout the early posthatching development, independently of phase of day (Figs. 1–2). Three types of HR fluctuations were dominant in developing chicks dependent upon the periodicity of baseline HR changes and the irregular occurrence of HR accelerations (Figs. 2 and 5). The first two patterns (Type I and Type II) had distinct oscillation frequencies. Type I fluctuations were defined by a widespread HR baseline and gives an impression of artifacts because two clear levels in HR are evident (Figs. 2–5). However, closer inspection of sequential points indicated that IHR oscillated over a range with fewer beats occurring at low HR levels. Type II fluctuations were predominated by cyclic oscillations in baseline HR with different frequencies. These Type I and Type II fluctuations may be categorized as HRV. Type III fluctuations were essentially non-cyclic, large accelerations and thus categorized as HRI. Not only did these HR fluctuations appear as single patterns, but also as combinations

of the three types in many of the individual chicks in daytime and at night (Figs. 3 and 4).

Type I and Type II HRV have not been found in prenatal embryos before pipping, but seemed to occur already in externally pipped (perinatal) embryos<sup>(28)</sup>. When the perinatal embryo began to breath air by the lungs, Type I HRV was recorded briefly at first and during a variable period later when the HR baseline was lowered<sup>(28)</sup>. During the EP period before hatching, two patterns of cyclic, accelerated HR fluctuations with different frequencies and durations were recorded; one at a relatively high frequency (ca. 5 times per min) and of low magnitude, lasting a relatively long period (ca. 20 min) (referred to as relatively long-lasting cyclic small accelerations), and the other at a relatively low frequency (ca. 2-3 times per min) and of large magnitude, lasting a short period (ca. 4-5 min)<sup>(28)</sup>. The former, relatively long-lasting cyclic small accelerations, seems to be predecessor of Type II HRV in newly hatched chicks in terms of frequency and magnitude. The latter is a unique pattern observed only in the perinatal embryos just prior to hatching and was not recorded in chicks after hatching.

The rapid accelerations with slower recovery to HR baseline already occurred in prenatal embryos<sup>(1,3,11,28)</sup>. The accelerations began to occur on around day 15-16 of incubation with a magnitude which was small at first and then augmented toward the pipping period. These spontaneous, irregular acceleration patterns, which may be the predecessor of Type III HRI in chicks, increased in magnitude further during the EP period<sup>(28)</sup>.

In prenatal embryos, HR fluctuations were also characterized by transient, rapid decelerations of HR<sup>(1,3,11,28)</sup>, which appeared after around day 13-14 of incubation and often occurred repetitively during late incubation. But, these characteristic transient decelerations of HR were rarely recorded in newly hatched chicks compared with prenatal embryos.

#### 4.2 Origins of heart rate fluctuations

Spectrum analysis of HR fluctuations in newly hatched chicks revealed two dominant frequency ranges with means at  $0.742 \pm 0.229$  Hz ( $N = 51$ ) and  $0.069 \pm 0.015$  Hz ( $N = 27$ ). These two frequency ranges resulted in characteristic oscillation patterns, which were defined as Type I and Type II HRV (Fig. 2). Literature discussing HR fluctuations in mammals is extensive<sup>(4,5,9,18)</sup>, but almost completely lacking for birds. Three frequency ranges for HR fluctuations have been defined as high frequency (HF), medium or low frequency (LF) and very low frequency (VLF). HF is centered at the ventilation frequency of each species, whereas LF and VLF occur at lower, variable frequencies dependent on the species investigated. In the present study on chick HR, the HF component is considered to be

equivalent to the Type I HRV, as was confirmed by additional experiments with visual counting of ventilation frequency, that is, respiratory arrhythmia, although the structure and breathing movements in avian respiratory system are different from those in mammals. The origin of the Type II HRV at a lower mean frequency of 0.069 Hz is problematic. In mammalian studies, the LF is attributed to the baroreflex loop's influence on HR mediated by both sympathetic and vagal activities<sup>(4,5,9)</sup>. The VLF is thought to be related to thermoregulatory fluctuations in vasomotor tone<sup>(4,5)</sup>. If Type II HRV is LF, that is due to baroreflex activity, then its influence on HR would be expected to be rapid. In the case of the rat, with a mean HR of 337 bpm, a LF of 0.34 Hz was reported<sup>(9)</sup>, and therefore the oscillation period is calculated to be within 17 beats. Type II HRV in this study had an oscillation period of 14.5 sec on average (MHR = 315 bpm), equivalent to 76 beats. Therefore the response time appears to be too slow for effective baroreceptor mediation of HR compared with the rat. Whether the Type II HRV is attributed to VLF component is unclear. The origins of Type II HRV remain to be studied, and administration of pressor or depressor drugs through a catheter implanted in a blood vessel or subcutaneous injection may elucidate whether or not Type II rhythms are mediated by baroreceptors.

Continuous measurements of IHR revealed that HR fluctuation patterns were changeable with time (Fig. 4). It is noteworthy that at the peak of HR accelerations (e.g., Type II in Fig. 3B), Type I HRV disappeared and then reappeared as IHR decreased again. Additionally, as shown in Figure 4, Type I HRV was correlated with the decreases in baseline HR and it intermittently disappeared during transient accelerations in both Type II and Type III fluctuations. Type I HRV tended to occur when chicks were quiescent and sleeping and was attenuated when they were active and calling. It was reported that administration of atropine to late chick embryos blocked the transient HR decelerations that occurred frequently and elevated HR baseline, implying that parasympathetic nervous functions play a role in regulating the HR in late embryos<sup>(11)</sup>. The parasympathetic tone seems to be also significant in chicks after hatching, even though transient decelerations were not present frequently, resulting in respiratory arrhythmia (Type I HRV) during periods of depressed HR. Although the sympathetic nervous system is considered to mediate the transient accelerations in prenatal embryos and Type III HRI in chicks, evidence for its role is still unclear<sup>(11)</sup>.

#### 4.3 Diurnal rhythms

Chick IHR showed a distinct diurnal rhythm (circadian rhythm), which was minimal during the night and on average maximal during



the daytime (Figs. 6 and 7). Baseline HR was relatively stable at night as chicks were most likely sleeping. However, baseline HR changed rapidly and frequently during the daytime by as much as 200 bpm (Fig. 6). These minima in HR during the daytime were similar to the baseline HR during the night on days 0 to 2 in some chicks, but from day 3 to 6 minima during the daytime were generally higher than at night in all chicks. If these minima reflect periods of inactivity, then the significantly elevated HR minima during the day are probably correlated with the diurnal rhythm in metabolism and the influence of freely available feed<sup>(19)</sup>. Conversely, sustained increases in HR probably reflect periods of activity, as chicks could move in the cage during measurements. Both minima and maxima in HR increased on early days after hatching, but maxima increased more than minima. The scope of HR changes (maxima-minima) was less than 50 bpm on days 0-2 and up to 250 bpm on days 5-6 in comparison to about 30 bpm and 100 bpm, respectively, for similar developmental times reported previously<sup>(19)</sup>. The lower scope of HR of the latter study is considered to be due to the counting of heart beats for only 10 sec in every minute<sup>(19)</sup>, which probably reduced the variability in their records. The higher daytime HR of chicks in the present study in comparison to the previous non-invasive studies in our group<sup>(24,29)</sup>, which restrained the chicks in a measuring device, limiting physical movements, also suggests that the occasional HR maxima that were recorded here were attributed to chick activity.

#### ACKNOWLEDGMENT

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## 成長するニワトリ雛における心拍リズム

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## 概要

ニワトリ雛の瞬時心拍数を孵化した日から6日目まで、無侵襲測定した心電図より決定した。実験 I では、心拍ゆらぎ（変動性と不規則性）を明らかにするため、瞬時心拍数を一日に昼間と夜間の二回それぞれ一時間計測した。雛の瞬時心拍数は本質的に変動しており、100-150 回/分の頻脈が支配的である。これらの心拍ゆらぎを三つのタイプに分類した；1) タイプ I：平均振動周波数 0.74 Hz (0.4-1.2 Hz の範囲) を持つ呼吸性不整脈による幅広い (20-50 回/分) ベースライン心拍ゆらぎ、2) タイプ II：平均 0.07 Hz (0.04-0.1 Hz の範囲) で心拍ベースラインが変動する低周波数振動、及び 3) タイプ III：頻繁に一過性に起きる非周期性のゆらぎ。実験 II では、孵化後一週間にわたって自然の昼夜周期、負荷とならない環境温度及び自由に採れる食餌条件で瞬時心拍数を測定した。心拍数は採餌と活動により昼間は非常に変動 (200-250 回/分) し、夜間は平均の心拍数は安定かつ減少して、日内リズムが現れた。心拍ゆらぎは昼夜を通して見られた。

キーワード：雛、日内リズム、心電図、心拍ゆらぎ、心拍不規則性、心拍変動性、瞬時心拍数、スペクトル解析

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