Cardiac rhythms in developing emu hatchlings[☆]

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Abstract

Six emu hatchlings were non-invasively measured for electrocardiogram (ECG) from their chest wall using flexible electrodes, and the instantaneous heart rate (IHR) was determined from ECG throughout the first week of post-hatching life. Although the baseline heart rate (HR) was low, approximately 100–200 beats per min (bpm), compared with chick hatchlings, the IHR fluctuated markedly. The fluctuation of IHR comprised HR variability and irregularities that were designated as types I, II and III in chick hatchlings and additional large accelerations distinctive of emu hatchlings. Type I was HR oscillation with a mean frequency of 0.37 Hz (range 0.2–0.7 Hz), i.e. respiratory sinus arrhythmia (RSA). From RSA, breathing frequency in emu hatchlings was estimated to be approximately half of that in chickens. Type II HR oscillation was also found in the emu; the frequency ranged from approximately 0.04 to 0.1 with a mean of 0.06 Hz, and the magnitude tended to be large compared with that of chickens. In addition to type III HRI, which was designated in chickens, large, irregular HR accelerations were characteristic of emu hatchlings. From IHR data, developmental patterns of mean heart rate (MHR) were constructed and plotted on a single graph to inspect the diurnal rhythm of MHR by visual inspection and power spectrum analysis. A circadian rhythm was not clear in the emu hatchlings, in contrast to chick hatchlings, which showed a dominant diurnal rhythm. © 2002 Elsevier Science Inc. All rights reserved.

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1. Introduction

The emu (*Dromaius novaehollandiae*) and ostrich (*Struthio camelus*) have become commercially farmed animals in several countries throughout the world for the promotion of meat supply; multi-functional oil is also produced from the emu. Eggs of these largest and second-largest birds weigh approximately 1400 g for the ostrich and approximately 600 g for the emu. Daily changes (developmental patterns) in embryonic mean heart rate (MHR) of these large ratites are different from those of other birds during the second half of incubation. In general, small altricial birds tends to increase their embryonic MHR towards hatching, and the maximum values are attained during the pipping (perinatal) period of incubation (Burggren et al., 1994; Tazawa et al., 1994; Pearson and Tazawa, 1999a,b; Pearson et al., 1999). In the meantime, the general developmental patterns of embryonic MHR in precocial birds are likely to be convex, i.e. the maximum values are attained during the prenatal (pre-pipping) period (Tazawa et al., 1991; Pearson et al., 1998; Tazawa et al.,

1998a,b, 2000). Particularly in the emu and ostrich, embryonic MHR seems to become maximum during the middle of incubation (Tazawa et al., 1998a,b, 2000). It was found that the MHR at 80% of incubation duration in precocial birds and that during the pipping period in the altricial birds have an allometric relationship with their fresh egg masses with a power of -0.123 (Tazawa et al., 2001).

While cardiac rhythms are expressed by MHR over a certain period of time, they are also expressed by beat-to-beat changes, that is, instantaneous heart rate (IHR). Although embryonic MHR has been determined for many species of birds, the development and behavior of embryonic IHR were studied to a limited extent in the domestic fowl (*Galllus gallus domecticus*) (Akiyama et al., 1997, 1999; Höchel et al., 1998; Tazawa et al., 1999; Moriya et al., 2000) and the muscovy duck (*Cairina moschata f. domestica*) (Höchel et al., 1999). In a companion report, the IHR fluctuations of emu embryos were investigated (Kato et al., 2002). For hatchlings, brief measurement of IHR was attempted in king quail (Pearson et al., 1998). In the domestic fowl (referred to as chicken or chick), the development of IHR fluctuations, as well as the developmental patterns of MHR, has been investigated in hatchlings (Moriya et al., 1999, 2000). Distinctive patterns of IHR fluctuations, HR variability (HRV) and HR irregularities (HRI), were found in chick hatchlings and were categorized into three types (Moriya et al., 1999). Type I HRV is a wide baseline HR, which corresponds to respiratory sinus arrhythmia (RSA) with a mean oscillating frequency of 0.7 Hz (range 0.4–1.2 Hz); type II HRV comprises HR oscillation with a mean of 0.07 Hz (range 0.04–0.1 Hz); and type III HR fluctuation is characterized by non-cyclic irregularities caused by HR accelerations. In addition, the baseline HR tends to increase during the first week after hatching, with significantly higher daytime HR than nighttime HR, exhibiting a distinct circadian rhythm.

In the present report, we investigated whether these IHR fluctuations and circadian rhythms occur in emu hatchlings. The cardiac rhythms in emu hatchlings are compared with those previously obtained from chick hatchlings (Moriya et al., 1999).

2. Materials and methods

Eggs were first incubated in a forced draught incubator at a temperature of 36 °C and relative humidity of 30% at the Cross Timbers Emu Ranch in Flower Mound, Texas, where they were collected. After several weeks of incubation there, eggs were transported to a laboratory in the University of North Texas and incubation was continued in another forced draught incubator at the same temperature and humidity. Egg turning was automatically carried out every 3 h daily in both incubators.

When an emu hatched, the feathers at the lateral thoracic wall under both wings and on the ventral abdomen, caudal to the sternum, were shaved to allow for the placement of three electrodes to measure electrocardiograms (ECG). The electrode was a flexible Ag/AgCl disk and attached to the skin by adhesive gel, as used for chick hatchlings (Moriya et al., 1999). The hatchling with electrodes attached was then transferred to a ventilated experimental box maintained at 36 °C. Light conditions were set to a 12 h/12 h light/dark cycle. The first day after hatching was designated as day 0. Hatchlings survived on their internal yolk until day 4, and food and water were supplied ad libitum from day 4.

The electrodes were connected to thin shield wires, which were fixed above the back of the hatchling with adhesive tape so that it could move freely in the experimental box. The electrode wires were then connected to a polygraph amplifier and ECG measurement was continuously made for 1 week after hatching. IHR was determined from the amplified and bandpass-filtered ECG signals with the aid of a computer. They were first sampled at a frequency of 2000 Hz using a Powerlab analog-to-digital converter. The sampling frequency of 2000 Hz could determine IHR with an error of less than 1 bpm for emu hatchlings, for which HR was low compared with chick hatchlings. The ECG signals sampled were compared with a threshold level that was set above background noise levels to determine the time at which the rising deflection of the QRS complex exceeded the threshold. IHR (in bpm) was then calculated from the time interval between the adjacent QRS waves.

In order to determine whether the IHR fluctuations were cyclic or not and the oscillatory frequency when they oscillated, time sequence IHR data during 20-min periods were analyzed for their

power spectrum using a fast Fourier transform (FFT), as reported elsewhere (Akiyama et al., 1999; Moriya et al., 1999). IHR data given by a series of points with equal time intervals were divided every 512 points from the beginning of 20-min recording and then from the first 257 points of the same 20-min recording. IHR data of each section comprising 512 points were calculated by FFT for power spectrum. The spectra of all the sections were averaged in order to improve the resolution of the spectral peak. Using the average method, we did not window the data. The power in the various frequency bands was described in normalized units. The frequency band we analyzed ranged from approximately 0.01 to approximately 1 Hz. The upper range depended on the mean value of HR.

In order to identify whether circadian rhythm of HR occurred by visual inspection, MHR over 1-min intervals was determined from IHR and the continuously measured HR data were presented on a single graph. When the visual inspection identified the possibility of circadian rhythm, daily change (i.e. developmental pattern) in MHR was analyzed by the following sine function:

$$MHR = |A + B \cdot \mathbf{x}| \cdot \sin[2\pi f \cdot \mathbf{x}] + C \cdot \mathbf{x} + HR_{ini} \qquad (1)$$

where **x is age** (in day), A (in bpm) is the amplitude of the circadian rhythm, B (in bpm per day) is the daily change in amplitude, f (in day^{-1}) is the frequency of the circadian rhythm, and its inverse, T (in days) $= 1/f$, is the period of the circadian rhythm, C (in bpm per day) is the daily change in MHR and HR_{init} (in bpm) is the y-axis intercept.

3. Results

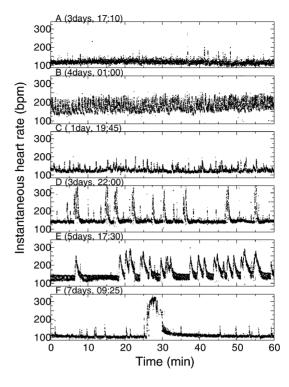### 3.1. Instantaneous heart rate fluctuations

Six hatchlings were subjected to continuous measurement of IHR. The HR was substantially arrhythmic, and HR accelerations were predominant throughout the first week after hatching. Various patterns of IHR fluctuations occurred in all individuals, independently of age, and are shown in Fig. 1. Examples are presented that correspond to type I, II and III HR fluctuations designated in chick hatchlings. Fig. 1A,B,C correspond to type I, type II and type III HRI, respectively. The HR accelerations shown in Fig.

Fig. 1. Examples of instantaneous heart rate (IHR) fluctuations presented by 60-min recordings. The age of hatchlings and time of measurement are shown in the parentheses of individual panels. The recordings were taken from six different individuals. (A) Wide heart rate (HR) baseline (Type I HRV) with sporadic episodes of HR accelerations; (B) HR oscillation (Type II HRV); (C) HR irregularities (Type III HRI). (D–F) Large HR irregularities distinctive of emu hatchlings.

1D,E,F are distinctive of emu hatchlings and may be categorized as new type III HRI for the emu.

In order to identify various patterns of IHR fluctuations by visual inspection and power spectrum analysis, individual 20-min segments were extracted from the 60-min recordings of Fig. 1 and were analyzed for power spectrum (Figs. 2–4).

Fig. 2 presents 20-min and 1-min recordings of the wide HR baseline extracted from Fig. 1A and normalized power of various frequency bands calculated for the 20-min recording. The 1-min recording shows a periodic HR oscillation with a magnitude of approximately 20 bpm and spectrum analysis presents a peak of power at 0.30 Hz (3.3 s). The HR oscillation that was shown as the wide HR baseline in a long-term recording frequently occurred in all hatchlings. For 65 examples taken at random from all hatchlings, the frequency was 0.37 ± 0.12 (S.D.) Hz with a range of 0.2–0.71 Hz. The amplitude was approximately 10–30 bpm.
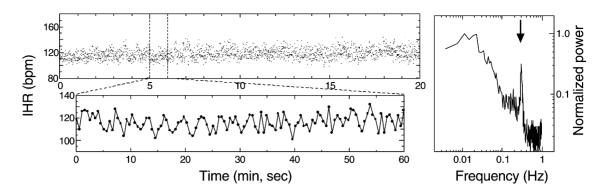
Fig. 2. A 20-min recording of instantaneous heart rate (IHR) extracted from Fig. 1A (left top panel), 1-min recording extracted from the above 20-min recording (left bottom panel) and normalized power analyzed for the 20-min recording of IHR. Visual inspection of the 20-min recording does not identify the HR oscillation, but a wide HR baseline is still evident. The 1-min recording shows that IHR oscillates with an oscillatory frequency of 0.30 Hz, as indicated by an arrow in the power spectrum analysis.

Fig. 3 shows two examples of type II HRV and their spectrum analysis. The top 20-min recording was extracted from Fig. 1B. The HR oscillated at a frequency of approximately 0.06 Hz (16.7 s) and a magnitude of more than 50 bpm. The bottom 20-min recording is another example of type II HRV with a frequency of 0.04 Hz (25 s) that lasted approximately 12 min. HR oscillations for a short period, as shown in Fig. 1B, frequently occurred in all hatchlings. For 46 examples taken randomly from all hatchlings, the frequency was 0.06 ± 0.02 (S.D.) Hz with a range of approximately 0.04–0.1 Hz.

Fig. 4 shows four 20-min recordings of HRI extracted from Fig. 1C–F and their power spec-

trum analyses. Fig. 4a presents transient HRI with a duration of less than 10 s, which was categorized as type III HR fluctuation in chick hatchlings. The three patterns of HRI shown in Fig. 4b–d were distinctive of emu hatchlings, and are presented in order of accelerated duration. The HR accelerations in Fig. 4b had a larger magnitude of approximately 150 bpm and a longer duration of approximately 1 min than the conventional acceleration patterns of Fig. 4a. Fig. 4c shows another pattern of HR acceleration with a long duration and accompanied by a wide HR baseline. The HRI in Fig. 4d was accelerated to more than 200 bpm for a duration of approximately 4 min.
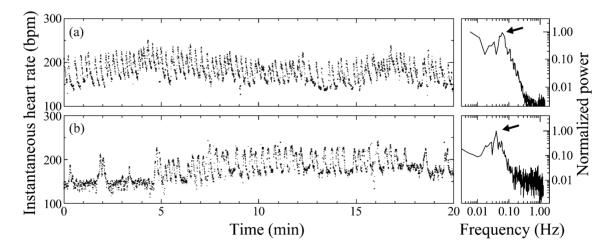

Fig. 3. A 20-min recording of instantaneous heart rate (IHR) extracted from Fig. 1B (left top panel) and another example of heart rate (HR) oscillation taken from the same hatchling at a different time (left bottom panel). The right two panels show normalized power analyzed for the individual left 20-min recordings. The approximate frequency for the peak of the power indicated by arrows is 0.06 and 0.04 Hz for (a) and (b), respectively.
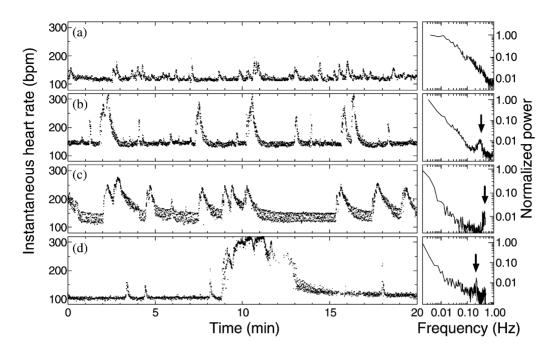
Fig. 4. The 20-min recordings of instantaneous heart rate (IHR) extracted from Fig. 1C–F and normalized powers for individual left recordings of IHR. Panels (a–d) correspond to Fig. 1C–F, respectively. (a) Brief, transient HR accelerations as shown in chick hatchlings; (b–d) large HR accelerations with long duration, which were not observed in chick hatchlings. The HR accelerations shown in (b–d) were accompanied by a wide HR baseline; that is, HR oscillation, as indicated by arrows in their power spectrum analyses. The oscillatory frequency was 0.31, 0.43 and 0.22 Hz, respectively.

Fig. 5 presents additional distinctive patterns of IHR fluctuations that occurred sporadically in emu hatchlings, but did not occur in chick hatchlings. Fig. 5a shows HR fluctuations that periodically accelerated with a frequency of approximately 0.07 Hz (14 s) for a duration of approximately 2 min at intervals of 2–3 min. Fig. 5b shows IHR that

continuously accelerated for approximately 1 min in duration and occurred at various intervals.

3.2. Developmental pattern of mean heart rate

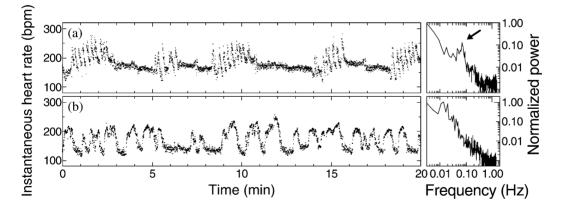Developmental patterns of MHR in six hatchlings were constructed from IHR data, and visual

Fig. 5. The distinctive, but rare patterns of heart rate fluctuations, which were not observed in chick hatchlings, and their spectrum analyses. The arrow in the right top panel shows a peak of approximately 0.07 Hz.
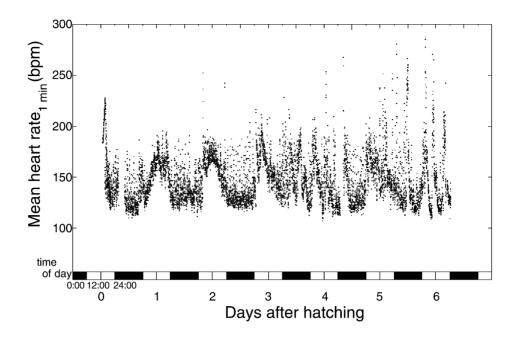
Fig. 6. The developmental pattern of mean heart rate (MHR) in an emu hatchling, which showed obvious difference in MHR between daytime and night.

inspection identified the possibility of circadian rhythm in two hatchlings, particularly in one embryo for which the developmental pattern of MHR is shown in Fig. 6. The open bar on the abscissa indicates the period from 06:00 to 18:00 h and the closed bar, the period from 18:00 to 06:00 h on the following day. This individual hatched at approximately noon and the MHR exceeded over 200 bpm during the first 3–4 h of the post-hatching period, with subsequent decrease to approximately 130 bpm. During the first 3 days, the baseline HR was higher in the daytime than at night and seemed to oscillate around HR of approximately 150 bpm. However, afterwards, diurnal changes in baseline HR were not as clear between daytime and night as those of other hatchlings. The developmental pattern of MHR during the period from day 0 to day 3 in Fig. 6 was approximated by the following sine function:

$$MHR = |15| \cdot \sin[2\pi \cdot 1.098 \cdot x] + 148 \qquad (2)$$

where $|r| = 0.558$. The developmental pattern during the 1-week period in Fig. 6 was approximated by:

$$MHR = |8.4| \cdot \sin[2\pi \cdot 1.089 \cdot x] + 147 \qquad (3)$$

where $|r| = 0.257$. Although the frequency of HR changes remained at approximately 1 day^{-1}, the correlation coefficient became small. In other hatchlings, the frequency of HR changes was not calculated to be approximately 1 day^{-1}. Daily change in MHR, which corresponds to C in Eq. (1), was 0 and the y-axis intercept was approximately 150 bpm [i.e. HR$_{ini}$ = 147 bpm in Eq. (3)], indicating that the HR baseline did not show an increasing trend during the first week, as it did in chicks, and the average of baseline HR was approximately 150 bpm.

4. Discussion

4.1. Heart rate variability and heart rate irregularities

The HR of animals is an important physiological factor with regard to O_2 transport to tissues, because the HR determines O_2 consumption according to the O_2 pulse (O_2 consumption per single heartbeat) that is the product of the arterio-venous O_2 content difference and stroke volume. Therefore, with regard to O_2 consumption, it is better for the HR to be kept stable, because abrupt fluctuations in HR might adversely change the O_2 pulse. In chick embryos, the IHR begins to fluctuate with the appearance of brief, transient deceleration on day 13–14 of incubation; IHR

fluctuation is augmented with subsequent increase in its magnitude and frequency and additional accelerations towards hatching (Höchel et al., 1998; Tazawa et al., 1999). However, the arterial blood pressure fluctuates little compared with the large HR fluctuations (Höchel et al., 1998). This implies that the O_2 pulse changes little during large IHR fluctuations and their average may be substantial for O_2 consumption. The O_2 transport must be kept adequate, even during large fluctuations of IHR. Meanwhile, it seems that large HR fluctuations occur in healthy animals, and contrarily, HR fluctuations diminish when the animals are morbid, as shown in emu embryos (Kato et al., 2002). However, the physiological significance of IHR fluctuations remains to be elucidated, and we are attempting to measure and collect IHR fluctuations in different species for comparison.

In chickens, embryonic HR fluctuations became maximum during the perinatal period and HR fluctuations were further augmented after hatching (Moriya et al., 1999, 2000; Tazawa et al., 1999). This trend was further emphasized in emu hatchlings with regard to patterns and magnitude. Although it was difficult to determine the baseline HR because of large HR fluctuations, the visual inspection identified it to be between 100 and 150 bpm in most emu hatchlings. The baseline HR was obviously low compared with that of chick hatchlings, although it was furthermore difficult to determine the baseline HR in chick hatchlings, which markedly changed within a day and also between days. In chick hatchlings, the IHR fluctuations were categorized into three types (Moriya et al., 1999).

Type I was HR oscillation with a period of approximately 0.8–2.5 s and a magnitude of 20–50 bpm, which was manifest as a wide HR baseline in a single graph of long-term recording. It was confirmed that type I HRV was caused by breathing and was respiratory sinus arrhythmia (RSA). Type I HRV was also recorded in emu hatchlings, as shown in Fig. 1A and in Fig. 2. The period of this HR oscillation was 3.3 s. In many other examples, the period ranged from approximately 1.4 to 5 s with a mean of 2.7 s, which was almost double that in chickens. The emu hatchlings breathed more slowly than the chicks. The amplitude did not exceed over 30 bpm, which was smaller than that of the chick hatchlings, probably because of the low baseline HR. Type I HRV occurred very frequently with other types of HR

fluctuations, particularly with type III HRI in emu hatchlings, as in chick hatchlings. RSA occurred very frequently, both in the emu and the chicken.

Type II was also HR oscillation, but with a period of 10–25 s and a magnitude of approximately 50 bpm in newly hatched chicks. In emu hatchlings, similar HR oscillation occurred, as shown in Fig. 1B and in Fig. 3. Two examples of type II HRV in emu hatchlings had a period of 25 and 17 s, respectively, with an amplitude of approximately 70 bpm. The period was not constant, even in the same individuals, and ranged from approximately 10 to 25 s with a mean of 16 s. Type II HR oscillation occurred less frequently and for short periods in emu compared with chick hatchlings. In newly hatched chicks, it often occurred for several hours continuously, particularly in a low temperature environment. When the chick hatchlings that were acclimated to a high environmental temperature were exposed to a low temperature, their HR baseline increased and began to oscillate with a frequency corresponding to type II HRV. Inversely, transfer of chick hatchlings from a low to a high temperature environment eliminated type II HR oscillation (Tazawa et al., 2002). From these experimental results, type II HR oscillation was inferred to originate from the thermoregulatory function of newly hatched chicks. However, such an experiment was not carried out in emu hatchlings.

Type III was characterized by non-cyclic irregularities of IHR, dominated by brief, transient accelerations of various amplitudes. The brief duration of HR acceleration did not exceed 30 s and the maximum amplitude was not more than 100 bpm, with rare exceptions reaching 150 bpm in newly hatched chicks. Type III HRI also frequently occurred in emu hatchlings with various patterns (Fig. 1C–F and in Fig. 4). The amplitude ranged around 100 bpm as in chickens, but frequently exceeded its range and reached 150–200 bpm with prolonged duration. The brief accelerations ended within 30 s, but in contrast to chickens, some patterns of HR acceleration lasted for more than 2 min. The duration of HR acceleration shown in Fig. 4d extended over 4 min. This acceleration pattern might be formed by successive occurrence of a few of the single, large accelerations shown in Fig. 4b,c. Type I HRV simultaneously occurred with this type III HRI in many cases.

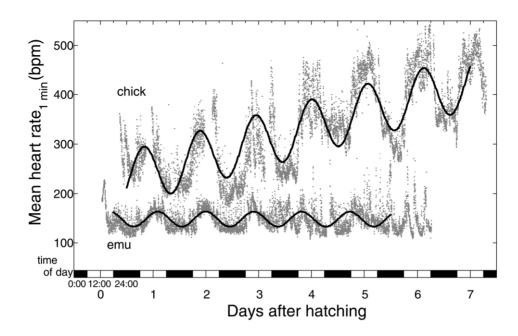Besides these IHR fluctuations as designated in newly hatched chicks, other rare patterns of HR

Fig. 7. Developmental patterns of MHR during the first week in a chick hatchling and the emu hatchling shown in Fig. 6, which were approximated by a sine function (solid curves).

acceleration were recorded in emu hatchlings, as shown in Fig. 5. Fig. 5a shows short-term repeated large accelerations of IHR, which occurred only sporadically in the three emu hatchlings. It may be inferred that the HR oscillation shown in Fig. 3 repeatedly occurred for a short period of 2–3 min. The IHR fluctuations shown in Fig. 5b were barely observed in only one emu hatchling. This pattern might be formed by the successive occurrence of several brief accelerations. However, this is speculation and the origins are unknown. In animals from fish to humans, beat-to-beat changes in HR are known to be mediated by the autonomic nervous system and reflect the activity of the reflex mechanisms involved in cardiovascular control (Altimiras, 1999). The IHR fluctuations shown in developing embryos and bird hatchlings must also be related to development of the autonomic nervous system. An investigation into the origins of IHR fluctuations was not within the scope of the present measurements, and remains to be studied.

4.2. Circadian rhythm of heart rate

Visual examination of the developmental patterns of MHR identified that the HR baseline irregularly changed in most emu hatchlings, but the approximate level of the baseline was located within a range of 100–200 bpm and did not significantly change during the first week measured. In two hatchlings, visual inspection identified that the daytime level of HR baseline was higher than that at night during a few days of one week. However, approximation by sine function indicated that only one of them had a diurnal period (Fig. 6). Meanwhile, in chick hatchlings, the HR baseline was higher than that in emu and the level of the HR baseline increased during one week (Moriya et al., 1999). In addition, the level of daytime HR baseline was markedly higher than that of nighttime, indicating a clear circadian rhythm of HR (Moriya et al., 1999). Fig. 7 presents an example of the developmental pattern of MHR in a newly hatched chick, together with that of the emu hatchling shown in Fig. 6. The diurnal change in MHR in the chicken is approximated by the following equation:

$$\text{MHR} = |21 + 7.3 \cdot \mathbf{x}| \cdot \sin[2\pi \cdot 0.948 \cdot \mathbf{x}] + 30 \cdot \mathbf{x} + 202 \quad (4)$$

where $|r| = 0.844$. Eq. (4) is drawn on the developmental pattern of MHR in Fig. 7. In the chick hatchling, the daily change in magnitude [B in Eq. (1)] was not zero, but 7.3 bpm per day, implying that the magnitude of the circadian rhythm increased during the first week. In addition, the

MHR increased daily by 30 bpm [*C* in Eq. (1)]. Other chick hatchlings, for which the developmental patterns of MHR were previously determined, similarly showed a diurnal change in MHR, which tended to increase in level and magnitude with their development. Meanwhile, in the emu hatchling, daily changes in the magnitude of the diurnal rhythm and those in the level of MHR were small. The period of diurnal change was also different between the chick and the emu, at approximately 25 and 22 h, respectively. This difference is shown by different sine curves for the chick and emu in Fig. 7. The other emu hatchlings did not show clear a diurnal rhythm of MHR by visual inspection. Further investigation into the circadian rhythm of HR in avian hatchlings is needed.

Acknowledgments

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