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ニワトリ胚の心拍ゆらぎに関連した胚動脈圧信号の力学系理論による解析

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Dynamical Systems Analysis of Arterial Blood Pressure Signals in Relation to Heart Rate Fluctuations in Chick Embryos

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We attempted a new approach based on a modern dynamical system theory to reconstruct the arterial blood pressure signals in relation to heart rate fluctuations of developing chick embryos. The dynamical systems approach in general is to model a phenomenon that is presented by a single time series record and approximate the dynamical property (e.g., heart rate fluctuations) of a system based only on information contained in a single-variable (arterial blood pressure) of the system. The time-series data of the arterial blood pressure was reconstructed in 3-dimensional space to draw characteristic orbits. Since the reconstructed orbits of the blood pressure should retain information contained in the pressure signals, we attempted to derive instantaneous heart rate (IHR) from the reconstructed orbits. The derived IHR presenting HR fluctuations coincided well with the IHR obtained conventionally from the peak-to-peak time intervals of the maximum blood pressure. Movements of the reconstructed orbits of the arterial blood pressure in 3-dimensional space reflected HR fluctuations (i.e., transient decelerations and accelerations).

Keywords: arterial blood pressure, chick embryo, dynamical systems approach, heart rate fluctuations, instantaneous heart rate, orbit reconstruction, reconstructed orbit, 3-dimensional space

1 INTRODUCTION

Incubated avian eggs have been used as an experimental model for investigation of developmental physiology^(3,23). Physiological functions of embryos developing inside an eggshell are completely independent of maternal functions and some physiological variables can easily be measured while maintaining adequate gas exchange through the eggshell. Among those variables, embryonic heart rate of chickens (HR) is determined throughout nearly the whole period of incubation by detecting the cardiogenic signals with various invasive and noninvasive methods^(1,2,4,6,8-16,21,22,24). Catheterization of the allantoic artery, which is made by taking advantage of the eggshell, makes it possible to measure the blood pressure during the last half of incubation prior to pipping^(19-20,25,26). Allantoic arterial blood pressure was recently used to determine instantaneous heart rate (IHR) of developing chick embryos⁽⁷⁾. Blood

was determined from individual peak-to-peak pressure is a simple monophasic wave and IHR intervals of the wave. Various patterns of HR fluctuations were found in late embryos.

From a viewpoint of systems information engineering, the blood pressure wave composed of low-frequency signals and the derived IHR containing HR fluctuations seem to be a favorable biological model for analysis using modern dynamical systems theory (deterministic approach)⁽⁵⁾. The dynamical systems approach is to model irregularly time-varying phenomena (time-series data) and approximate the dynamical property of a system based only on information contained in a single variable of the system⁽¹⁷⁾. Although the theory of the dynamical systems approach has been tested in many artificial system models, it has seldom been applied to biological phenomena. Thus in relation to the symposium on cardiac rhythms in animals, we attempted to apply the dynamical systems approach to reconstruct the arterial blood pressure signals as an orbit in a high dimensional space and derive IHR from reconstructed orbits. In the present attempt, the single variable that is used in the dynamical systems approach is arterial blood pressure and the dynamical properties that are derived are the fluctuations of IHR.

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2 MATERIALS AND METHODS

2.1 Arterial blood pressure signals

In a previous study, the arterial blood pressure of the allantoic circulation was measured to derive IHR and investigate development of HR fluctuations in chick embryos⁽¹⁾. A catheter comprised of a hypodermic needle and polyethylene tube was implanted in the allantoic artery through a small opening in the eggshell. The catheter was fixed onto the eggshell with clay and the opening was closed with vinyl tape and epoxy glue. A conventional strain gauge manometer was connected to the catheter, and the pressure signals were amplified and filtered by a polygraph amplifier to match the input signal level of an analog-to-digital converter. The pressure signals were sampled at 100 Hz, recorded on a microcomputer, and restored by the following sinc function⁽²⁾:

$$BP(t) = \sum_{j=-\infty}^{\infty} BP(j \cdot I) \cdot \frac{\sin [2\pi f_N(t - j \cdot I)]}{2\pi f_N(t - j \cdot I)} \quad (1)$$

where I is the sampling interval (i.e., 10 ms), and f_N is Nyquist's reflection frequency (50 Hz).

Wave restoration by eq. 1 using 401 sampling points (i.e., $j = 200$) was examined to correspond to a signal sampled at 8,000 Hz (sampling time interval = 125 μ sec), ensuring calculation of IHR with an error in accuracy of less than 1 beat/min. After restoration of the systolic pressure wave, the maximum point was found in the restored wave, the time interval between the two adjacent maxima ($.t$ in sec) was determined, and IHR was calculated by $IHR = 60/.t$ (beats/min, bpm). In the present study, we used the arterial blood pressure signals that were measured previously as above. Prior to use of the previously measured pressure signals, we examined again the accuracy of the pressure signals, which were sampled at 50 Hz (instead of 100 Hz) and restored by eq. 1 with 401 sampling points. In the test experiment, the catheter was implanted in the allantoic artery of an 18-day-old embryo as mentioned above and the arterial pressure was sampled at a frequency of 8,000 Hz instead of 50 Hz. IHR was calculated from the time intervals between two consecutive peaks of maximum pressure (referred to as HR_{8000}). Then, single values from every 160 sampling points (which corresponded to pressure signal sampled at 50 Hz) were extracted, and the pressure wave was restored by eq. 1 with 401 sampling points from the extracted values. IHR was calculated for restored pressure waves in the same manner (referred to as HR_{resto}). It was found that HR_{resto} was consistent with HR_{8000} , ensuring the accuracy of the pressure signals that were previously measured at a sampling frequency of

100 Hz and restored by the sinc function.

2.2 Orbit reconstruction

In the dynamical systems approach, an orbit (attractor) of a single variable (i.e., blood pressure in the present study) is reconstructed (embedded) in a high dimensional (i.e., m -dimensional) space from the time-series data of the variable. The embedding dimension, m , should be no less than 3 for irregularly time-varying signals. In order to present a pattern of the orbit as a visible figure on a computer screen, we reconstructed the orbit in 3-dimensional space. For time-series data of a given blood pressure, $BP(t)$ ($0 < t < T$; T = one cardiac period), we assumed a suitable time delay τ , which is discretionary and determined empirically, then took two more values, $BP(t+\tau)$ and $BP(t+2\tau)$. These three values of blood pressure at time t were then put on individual axes of a 3-dimensional rectangular-coordinate, giving a vector with coordinates. An orbit of the vector was drawn by varying time t by 125 μ sec during one cardiac period with coordinates, thus giving a reconstructed orbit of blood pressure during the T -period. The 3-dimensional orbits were drawn consecutively for time-series data of the blood pressure during nT period where n is discretionary real number. The presentation of orbit patterns was made only for a part of the pulse pressure (i.e., during nT period), and the position of 3-dimensional rectangular-coordinate axes was arbitrarily drawn to present differences in orbit size between young and late embryos, and changes in orbits during the heart beat of individual embryos.

2.3 Derivation of IHR from reconstructed orbits

The reconstructed orbit is another presentation of blood pressure and must include information contained in the time-series signal of blood pressure; that is, IHR also should be included. Thus we attempted to derive IHR from the reconstructed orbits. If the time delay τ is considered adequately; empirically a value within one-tenth of the cardiac period T , a vector comprising of $[BP(t), BP(t+\tau), BP(t+2\tau)]$ rotates in the 3-dimensional space during one cardiac period and forms an open or closed orbit. When the orbit is not closed, it forms a thin sheet-like configuration during nT period. Suppose a plane crosses at right angles to the sheet-like orbits at an arbitrary position in space. Then the time interval between points of the orbits which cross the plane in sequence is determined successively (i.e., $.t$ in sec), and the HR is calculated by $HR=60/.t$ in bpm and plotted on the computer monitor sequentially.

3 RESULTS

Figure 1 shows the arterial blood pressure of a 12-day-old embryo (panel A) and its reconstructed orbits (strange attractor)

drawn in 3-dimensional space (panel B). Blood pressure is presented for 30 min, and pressure

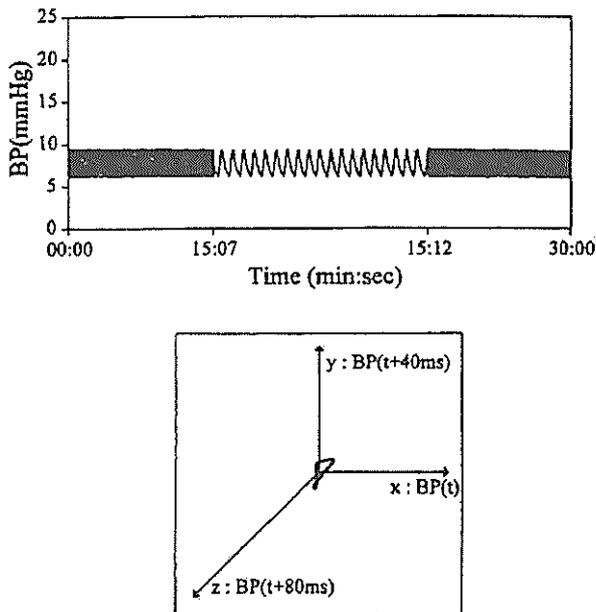


Fig. 1. Time-series record of the arterial blood pressure (top panel) and 18 orbits reconstructed in the 3-dimensional space (bottom panel). The embryo was 12-day-old.

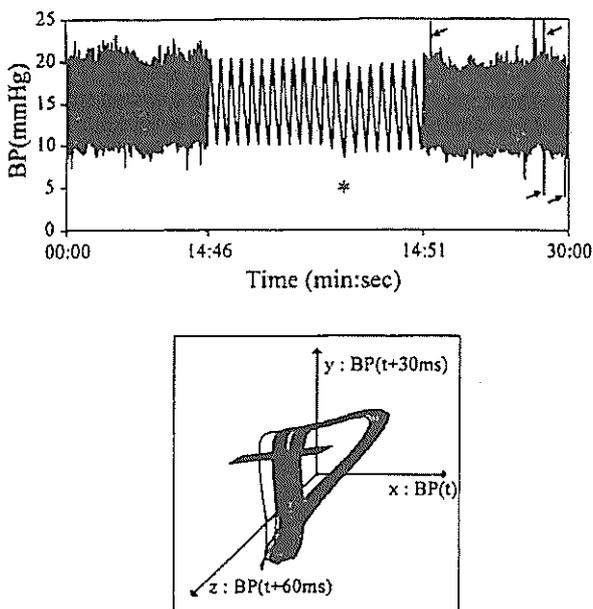


Fig. 2. The arterial blood pressure (top panel) and reconstructed orbits (bottom panel) in an 18-day-old embryo. For comparison between Figs. 1 and 2, the scales for the blood pressure record and reconstructed orbits are identical in both figures.

waves during a 5-sec period are shown in the middle of the recording at a faster chart speed (panel A). In young embryos, the blood pressure was low compared with late embryos and

formed simple monophasic waves. A single orbit was reconstructed from a blood pressure wave of one-cardiac period, and panel B presents 18 reconstructed orbits which correspond to the pulse pressure waves presented in the middle of panel A. The time delay (τ) of 40 msec was taken arbitrarily so that the orbits could make characteristic figures.

Figure 2 shows another example of the arterial blood pressure of an 18-day-old embryo (panel A) and its reconstructed orbits (panel B). Arterial blood pressure increased with embryonic development, but the waves were still monophasic (panel A). As embryos grew, their activities increased and induced artefacts in the pressure signals; i.e., abruptly recorded spikes marked by an arrow. Beside these artefacts, the blood pressure signals fluctuated as shown throughout and in the middle of the recording. The reconstructed orbits were drawn by taking arbitrarily τ of 30 msec (panel B) for 19 pressure waves during the 5-sec period (14:46-14:51 in panel A). The orbits moved towards the left along the x-axis with heart beats and reached the left most position at the 12th beat, which lowered the minimum pressure (as shown by an asterisk in about the middle of recording in panel A) and thereafter orbits moved towards the right again.

Because a single orbit is reconstructed from a pulse pressure wave during one cardiac period, duration of the cardiac period is known by cutting the orbits by an arbitrary plane (as shown by a thick line in panel B of Fig. 2) and measuring the time interval between points on the orbit crossing the plane successively. Then, the IHR in bpm is calculated from the time interval. Figure 3 presents the same arterial blood pressure as shown in Fig. 2 (panel A), IHR calculated conventionally from peak-to-peak time intervals of the blood pressure (panel B), and IHR derived from the reconstructed orbits as mentioned above (panel C) and 18 reconstructed orbits corresponding to a run of heart beats with a stable rate (panel D), decelerated rate (panel E) and accelerated rate (panel F), respectively. The IHR derived from the reconstructed orbits of the arterial pressure (panel C) coincides well with the IHR obtained by conventional calculation from the time interval of the maximum blood pressure (panel B). The reconstructed orbits for 18 cardiac periods during HR deceleration and acceleration present different changes in patterns and movements between them. When the HR is stable (marked by D in panel C, and panel D), the reconstructed orbits of the arterial pressure form a narrow band. When the HR decelerates, the orbits move leftwards along the x-axis, reach the left most position corresponding to the minimum of HR and then move towards the original position obtained for stable HR (i.e., towards the right) (marked by E in panel C, and panel E). Contrarily, the

reconstructed orbits move towards the right along the x-axis as the HR accelerates (marked by F in panel C, and panel F).

Figure 4 shows the arterial blood pressure recorded from a 20-day-old embryo (upper tracing in panel A), the IHR calculated from the time interval of the maximum blood pressure (lower tracing in panel A) and the reconstructed orbits (panels B, C and D) of blood pressure signals for the period indicated by the letters B, C and D in panel A. During a 33-sec recording, a single-beat acceleration and deceleration (B) and then an accelerated HR with single-beat deceleration (D) occurred. The reconstructed orbits present characteristic figures for these HR fluctuations. For 18 pressure signals, the reconstructed orbits present a similar pattern corresponding to stable HR, resulting in narrow band (marked by C in panel A, and panel C). The reconstructed orbits of 18 pressure signals marked by B in panel A constitute two narrow bands corresponding to two different levels of HR baseline, and the orbit moves posteriorly along the z-axis corresponding to HR acceleration and then soon moves anteriorly corresponding to subsequent deceleration. When the HR acceleration occurs during multiple heart beats (marked by D in panel A, and panel D), the orbits moving anteriorly constitute a wide band compared with the single acceleration (panel B). The subsequent single deceleration corresponds to the orbit, which moves anteriorly.

4 DISCUSSION

The beat-to-beat HR; i.e., IHR, in avian embryos developing inside the eggshell is determined from cardiogenic signals detected noninvasively or invasively^(2,7,12,24). Some of the cardiogenic signals used for IHR determination are ballistocardiogram (BCG), acoustocardiogram (ACG), electrocardiogram (ECG) and arterial blood pressure. IHR is calculated from the time interval between the two adjacent peak waves. The ECG which has sharp spike waves seems to be the most favorable cardiogenic signal for IHR determination. In avian eggs, the ECG is detected by electrodes implanted immediately inside the shell membrane or the chorioallantoic membrane in order to minimize injury to the embryo and egg contents. However, the ECG signal is often disturbed by embryonic activities, because the electrodes do not contact directly the body of the embryo, resulting in frequent interruption of IHR determination. Compared with the ECG and other cardiogenic signals, the blood pressure which is detected by the needle catheter implanted into the allantoic artery is less influenced by embryonic activities. Höchel *et al.*⁽⁷⁾ measured the blood pressure of the allantoic artery in chick embryos and presented the IHR by determining the time interval between the peaks of pressure waves

(i.e., maximum blood pressure). The question that arises is, in the event that the HR is

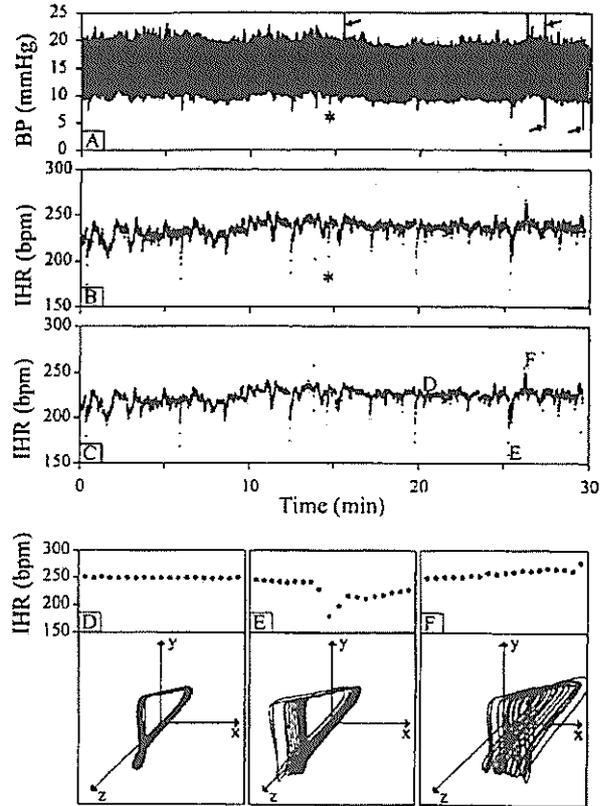


Fig. 3. The arterial pressure (BP, panel A), instantaneous heart rate (IHR) calculated from time intervals of the two adjacent peaks of blood pressure (panel B), IHR derived from the orbit reconstruction (panel C), and reconstructed orbits (panels D, E and F) corresponding to IHR indicated by the letters, D, E and F, respectively, in panel C. Time delay (τ) used for orbit reconstruction was 30 msec. Asterisk in panels A and B indicate the arterial blood pressure and IHR which correspond to the arterial pressure and reconstructed orbits shown in panels A and B of Fig. 2. Arrows in panel A are the same as in panel A of Fig. 1, indicating artefacts induced by embryonic activities. Other spikes of blood pressure in general seem to coincide chronologically with HR decelerations and accelerations.

calculated from the time interval between the minimum blood pressures or from the time interval between arbitrary levels of the pressure signals, is the same IHR obtained? The present determination of IHR from the reconstructed orbits showed that the IHR determined from the maximum blood pressure wave was the same as that derived from an arbitrary level of the pressure signals (Fig. 3). This suggests that even if the IHR is calculated from the minimum blood pressure, the same IHR is obtained and the conventional way of IHR calculation from the maximum pressure wave may be acceptable. Additionally, there is evidence that the IHR derived from the

reconstructed orbits coincides with the IHR obtained in conventional calculation from the maximum blood pressure signal, certifying that physiological information of the arterial blood pressure is retained in the reconstructed orbits.

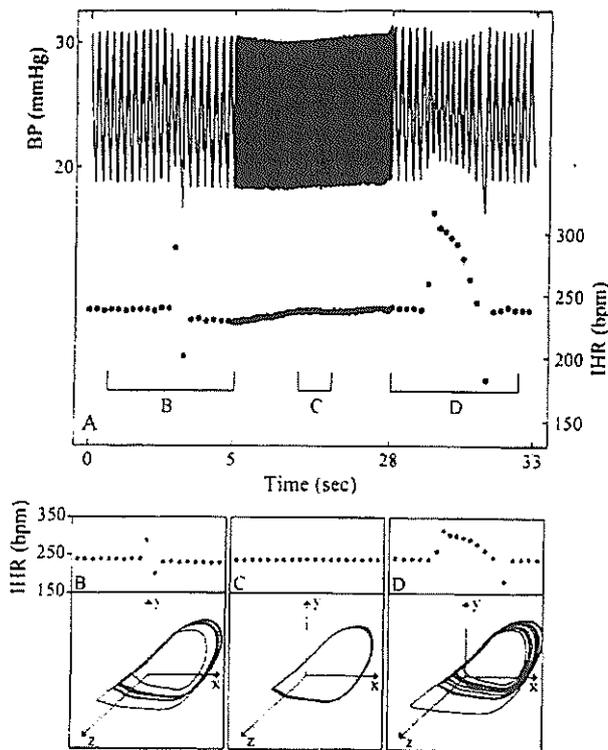


Fig. 4. The arterial blood pressure (upper tracing in panel A), IHR obtained from the pressure wave (lower tracing in panel A) and the reconstructed orbits (panels B, C and D) corresponding to the arterial blood pressure indicated by the letters, B, C and D, respectively, in panel A. Time delay (τ) was 30 msec.

In the dynamical systems approach, a time-varying single variable; i.e., the arterial blood pressure, $BP(t)$, is reconstructed by drawing of $[BP(t), BP(t+\tau), BP(t+2\tau), \dots, BP(t+(m-1)\tau)]$. In the present report, embedding dimension, m , of 3 was taken to show the orbit in a visible way (i.e., in figures). τ was determined empirically to within one-tenth of a cardiac period so that the orbit could show characteristic patterns. In other words, pattern of the orbit was not fixed, but changed by the value of τ . For reconstructed orbits of the blood pressures shown in Figs. 1 and 2, τ was taken arbitrarily as 40 msec and 30 msec, respectively, to show similar orbit patterns. In the young embryo (12 days), the magnitude of the reconstructed orbits was small compared with the late embryo (18 days), because the pulse pressure was low. In addition, the orbits formed a narrow band centered on a closed circle in the young embryo (panel B in Fig. 1) and the orbits in the 18-day-old embryo tended to move towards a

direction parallel to the x-axis, forming the orbits as a sheet-like configuration with a wide band (panel B in Fig. 2). The direction of movement and width of the band during several cardiac cycles reflect the HR fluctuations (Figs. 3 and 4). When the HR is stable, the orbits constitute a narrow band (panel D in Fig. 3 and panel C in Fig. 4). When the HR changes irregularly, the orbits form wide bands (panels E and F in Fig. 3, and panels B and D in Fig. 4). The HR fluctuations comprising transient deceleration and acceleration are presented by orbits moving in opposing directions corresponding to decelerated HR (panel E in Fig. 3) and accelerated HR (panel F in Fig. 3). In other examples of orbits representing a combination of a single acceleration and deceleration (panel B in Fig. 4) and sustained accelerations with single deceleration (panel D in Fig. 4), the orbits shrink along the z-axis corresponding to the HR accelerations and then widen when the single deceleration occurs. However, we do not know at present the physiological significance of these orbit patterns.

As far as the IHR is concerned, it is sufficient to determine IHR from the time intervals of the maximum blood pressure and thus record time-series data only for a part of the maximum pressure signals. Meanwhile, the IHR obtained from the chick embryos shows various fluctuation patterns that interest us as to whether they are deterministic phenomena. If we have time-series signals for the arterial blood pressure for the whole cardiac periods, the dynamical systems analysis (deterministic approach) can be attempted. In the present report, we could show characteristic figures of the reconstructed orbits for the arterial blood pressure of the embryos and derive IHR from the reconstructed orbits. Since this is the first attempt at using the dynamical systems approach, we assumed an embedding dimension, m , to be 3. The next step for orbit reconstruction should be to work on a higher dimensional space (i.e., m should be more than 3) and additionally a correlation dimension should be determined. Although in future studies additional calculations must be made to examine whether the HR fluctuations are chaotic phenomena, the HR fluctuations comprised of heart rate variability and heart rate irregularities will make favorable biological subjects for nonlinear time-series analysis.

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ニワトリ胚の心拍ゆらぎに関連した胚動脈圧信号の力学系理論による解析

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概要

成長するニワトリ胚の心拍ゆらぎに関連して動脈圧信号を再構成するために、力学系理論に基づく新しい解析を試みた。この解析法は、単一の時系列データによって表される一つの現象をモデル化し、任意のシステムの一つの変数（例えば、胚動脈圧）に含まれる情報のみに基づいてそのシステムの動的特性（例えば、瞬時心拍数）を推計する非線形法である。そこでまず、ニワトリ胚の動脈圧波形の時系列データを3次元空間に再構成した。血圧波形の再構成軌道は血圧信号に含まれる情報を保持しているはずなので、特徴的な再構成軌道を抽出して瞬時心拍数の時系列パターンを導出することを試みた。心拍ゆらぎを表す導出した心拍時系列パターンは、最大血圧のピーク間隔から従来の方法で得たパターンによく一致し、更に血圧波形の3次元空間再構成軌道の動きは心拍数ゆらぎを反映していることを示した。

キーワード：動脈圧、ニワトリ胚、力学系理論、心拍ゆらぎ、瞬時心拍数、軌道再構成、再構成軌道、3次元空間

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