Eur J Appl Physiol (2003) 89: 221–229
DOI 10.1007/s00421-002-0784-0

ORIGINAL ARTICLE

Kunihiko Nomura · Yoshiaki Takei
Yasuyoshi Yanagida

Comparison of cardio-locomotor synchronization during running and cycling

Accepted: 18 November 2002 / Published online: 28 February 2003

Abstract By comparing the characteristics of cardiac-locomotor synchronization (CLS) in running and cycling individuals, we tested whether the characteristics of CLS occurring during rhythmic exercise adhere to the central origin hypothesis, which postulates a direct interaction between cardiovascular centers in the brain and the pattern generator in the spinal cord. Ten healthy subjects performed both exercises at the same intensity (150 beats·min^{-1}) and cadence (150 steps·min^{-1} during running and 75 rpm during cycling), while electrocardiograms and electromyograms from the right vastus lateralis muscle were monitored continuously. An examination of the occurrence of heart beats with respect to the locomotor phase revealed that, in running subjects, CLS exists for relatively prolonged periods at specific phases, whereas, in cycling subjects, it occurs intermittently and is not phase-specific [maximum duration of CLS: 113.6 (66.5) and 58.0 (29.3) s ($P < 0.05$), respectively]. Determining the probability of CLS by chance as a function of its duration, we also found that, during running, CLS likely results from entrainment, whereas, during cycling, it results from chance, occurring when the cardiac rhythm approached the locomotor rhythm. Our result indicated that the duration of muscle contraction during cycling [317.0 (18.1) ms] was significantly longer than during running [205.6 (20.2) ms]. These results indicated that the difference in the CLS characteristics between running and cycling might be influenced by differences in peripheral inputs between exercise modes.

K. Nomura
Graduate School of Human Science, Kobe University,
3-11 Tsurukabuto, Nada-ku, 657-8501 Kobe-shi, Hyogo, Japan

Y. Takei (✉) · Y. Yanagida
Faculty of Human Development, Kobe University,
3-11 Tsurukabuto, Nada-ku, 657-8501 Kobe-shi, Hyougo, Japan
E-mail: takei@kobe-u.ac.jp
Tel.: +81-78-8037815
Fax: +81-78-8037815

Keywords Coupling · Cycling · Entrainment · Running · Synchronization

Introduction

The concept of "entrainment" or "synchronization" (Pavlidis 1973), wherein a nonlinear oscillator is entrained to an external oscillator when the frequency of external oscillator approaches that of the system, may be the basis of a general mechanism for heart rate (HR) regulation during the decrease in variability of HR. Such synchronization has been reported between the rhythms of biological oscillators that exhibit nonlinear behavior, in particular those that have relatively short periods, e.g., cardiac and locomotor synchronization (CLS) (Kirby et al. 1989b; Niizeki et al. 1993; Nomura et al. 2001), locomotor and respiratory synchronization (LRS) (Bechbache and Duffin 1977; Bernasconi and Kohl 1993; Paterson et al. 1986) and cardiac and respiratory synchronization (CRS) (Kenner et al. 1976; Schäfer et al. 1998; Seidel and Herzel 1998). During exercise, the increase in HR, which is important for blood circulation, results from the increase in cardiac sympathetic nervous activity and the withdrawal of cardiac vagal nervous activity. The transmission of an efferent signal via the sympathetic nervous system is, however, too slow to regulate cardiac rhythm on a beat-by-beat basis (Berger et al. 1989).

Previous studies have shown that CLS occurs during walking, running and cycling (Kirby et al. 1989b; Niizeki et al. 1993; Udo et al. 1990). For example, Kirby et al. (1989b) found that the frequency ratio of cardiac and locomotor rhythms, which were determined from brief (ten steps during walking and running, 4 s during cycling) samples of data, became an integer with less than 1.0% difference with a spontaneous cadence during all three activities. Furthermore, some investigators have analyzed the dynamics of heartbeats with respect to the locomotor phase, namely the phase domain approach

(Niizeki et al. 1993; Nomura et al. 2001; Udo et al. 1990). Udo et al. (1990) showed that R waves repetitively occur at a specific phase within each step cycle during running, and Niizeki et al. (1993) showed phase locking between the cardiac and locomotor rhythms during both walking and running. These studies did not, however, address the question of whether CLS occurred as a matter of coincidence or whether there was a linkage between the two rhythms.

Kirby et al. (1991, 1992) tested whether the observed CLS during rhythmic exercise was statistically significant using a cross-over test in which they compared the cardiac rhythm of each subject to the locomotor rhythm of another age- and gender-matched subject. Since they did not analyze CLS with beat-by-beat and/or step-by-step analysis, the accidental possibility of CLS during skipping, hopping and finger tapping might not be rejected. Using beat-by-beat analysis of CLS and rejection of the null hypothesis that CLS reflects a chance intersection between two independent rhythms, we recently demonstrated that, during running, the phase relationship between cardiac and locomotor rhythms is likely a consequence of entrainment of one rhythm by another (Nomura et al. 2001). To characterize the relationship between cardiac and locomotor rhythms independently in that experiment, we generated surrogate data by means of a random shuffle of the series of gait cycle. In that case, if CLS occurred by chance, the surrogate data would have the same characteristics as the original in a phase domain. After surrogation, however, the phase relationship between the two rhythms was not indicative of CLS; in other words, the null hypothesis was rejected. It remains unclear whether, during cycling, the CLS occurring results from entrainment or by chance.

It has been proposed that the activity of neural circuits originating in the periphery (e.g., muscle afferents) (Niizeki et al. 1993) and in the central nervous system (Kawahara et al. 1993; Kirby et al. 1990) is the physiological process underlying CLS. Based on the analysis of phase response curves, Niizeki and Miyamotmo (1999) proposed that CLS has a peripheral origin and described the phase dependence of HR responses on the temporal relationship between muscle contraction and cardiac phase. They suggested, based on the concept of Pavlidis (1973), that because the relationship between the timing of muscle contractions and normalized heart beat intervals had a positive slope for the first quarter of the cardiac cycle and a negative slope for the remaining three-quarters of the cardiac cycle, muscle contractions occurring during the middle phase of the cardiac cycle might cause stable CLS. They further suggested that the physiological mechanism would involve a phase-dependent effect of afferent signaling from active muscle to the cardiovascular centers in the brain via group III fibers.

On the other hand, Kawahara et al. (1993, 1994) proposed that the modulation of heart beat rhythm by locomotor rhythm originates in the central nervous system. They described the existence of a distinct peak in coherence between HR variability and lateral gastrocnemius nerve discharge that corresponded to the mean stepping frequency in paralyzed, vagotomized, decerebrate cats during fictive locomotion elicited by activation of the mesencephalic locomotor region. The concept of a central origin is that there are interactions between the cardiovascular centers in the brain and the central pattern generator in the spinal cord, which generates locomotor patterns (Grillner 1975).

Even though running and cycling are different modes of exercise, they generate the same locomotor rhythm, and so, if centrally originating CLS exists in humans, it should have the same characteristics, including rejection of the null hypothesis mentioned above, during rhythmic exercises with the same work rate and locomotor rhythm. The aim of the present study was to determine whether the same characteristics of CLS are indeed observed during different modes of rhythmic exercise. To that end, we compared the phase relationship between cardiac and locomotor rhythms during cycling and running, which can be performed at the same HR level and locomotor rhythm, but represent different modes of exercise. We used the surrogate data technique (Nomura et al. 2001) and the phase domain approach to decide whether it resulted from entrainment or by chance, and used an index based on conditional probability (Stefanovska et al. 2000) to assess the strength of the CLS.

Methods

Subjects

Ten healthy men [mean age 22.9 (range 20–25) years] with no history of cardiopulmonary disease participated in the study. Each subject signed informed consent after being provided with a verbal explanation of the intent and procedures of the experiments. This study was approved by Human Subjects Committee of our department in Kobe University.

Protocols

A 5-min warm-up period was followed by at least 15 min of rest during which subjects were instrumented for data collection. Thereafter, each subject participated in a 20-min session in which he ran on a treadmill (Treadmill NT-12, Nishikawa) at 150 steps·min^{-1} or cycled on a cycle ergometer (STB-1400, Nihonkoden) at 75 rpm controlled by metronome at 2.5 Hz throughout the session. Subjects performed the two exercises in random order on non-consecutive days. Before the measurement period, a variety treadmill speeds or work rates were used in an attempt to explore the exercise intensity that produce the HR of 120 beats·min^{-1} while they practised running or cycling in time to the metronome at 2.5 Hz. For the first 5 min of the measurement periods, the subjects worked at a rate that produced a HR of 120 beats·min^{-1}. During the next 5–10 min, the treadmill speed or cycling work rate was gradually increased until a target HR (THR) of 150 beats·min^{-1} was achieved, which was then maintained for a minimum of 5 min. None of the subjects could maintain the maximum HR for 20 min. Furthermore, each subject had their own preferred locomotor cadence during running. When the pitch is controlled, the range of controllable pitch for a long period of running can be restricted within a narrow range. Thus, we set the level of HR at 150 beats·min^{-1} and the pitch at 2.5 Hz corresponding to 150 steps·min^{-1} in

order to solve both problems (i.e., pitch control and HR level). During that period, the treadmill speed ranged from 100 to 200 m·min^{-1} (0% grade), and the cycling work rate was ranged from 100 to 180 W.

Data collection

Beat-to-beat RR interval (RRI) was measured continuously from a surface electrocardiogram (ECG) using standard bipolar leads (CM_5). To distinguish the R waves of the QRS complex, the ECG signal was amplified, filtered (10–300 Hz) (AB-621G, Nihonkoden) and digitized using a 16-bit analog-to-digital converter (DR-Ma2, TEAC) sampling at 1000 Hz. To avoid movement artifact, we set filtering frequency band as 10–300 Hz. The data were stored on a MS-DOS-formatted magnetic optical disk for later analysis. A customized computer (PC9821Xc13, NEC) program detected the occurrence of the QRS spikes in the ECG at a sampling frequency of 1000 Hz.

The gait cycle (GC) was defined as the interval between the onset of one muscle contraction and that of the next. GC was measured continuously from a surface electromyogram (EMG) from right vastus lateralis muscle using bipolar leads. Surface electrodes were placed along the length of the muscle. The electrodes were placed at approximately 10 cm superior to the head of the fibula in order to avoid an artifact of EMG baseline by leg motion. The inter-electrode distance was about 30 mm, and the earth electrode was placed on the upper thigh. As with the ECG signal, the EMG signal was amplified, filtered (50–1000 Hz), digitized using the analog-to-digital converter and stored on a magnetic optical disk and processed off-line. To obtain an integrated EMG, the signal was full-wave rectified and smoothed. Because the EMG of the vastus lateralis muscle discharges mostly at the initial contact of the foot with the treadmill surface, the onset of muscle contraction was defined as the time at which the integrated EMG increased above a preset trigger level using the customized computer program.

Data analyses

The phase difference between cardiac and locomotor rhythms needs to be studied with beat-by-beat and/or step-by-step analysis over prolonged periods (Kirby et al. 1989b). Consequently, after removing a small number of abnormal RRIs and GCs, the occurrence of CLS was monitored using a phase synchrogram (Rosenblum et al. 1998; Schäfer et al. 1999). Each successive QRS spike and muscle contraction onset was marked as equivalent to one oscillatory cycle, to which a 2π increment was added. Within this one oscillatory cycle, the instantaneous phase is:

$$\phi(t) = 2\pi \frac{t-t_k}{t_{k+1}-t_k} + 2\pi k, \quad t_k \le t < t_{k+1}$$

where $\phi(t)$ is the instantaneous phase at t, and t_k is time of the kth marker event. In this definition, the phase is a monotonically increasing piecewise-linear function of time defined on a real line. The onsets of muscle contractions were taken for the marker events of the locomotor oscillator. The generalized relative phase ($\Psi_{RR\text{-}GC}$) of the GC for the occurrence of an R wave with respect to one GC was calculated as follows.

$$\Psi_{RR-GC} = \frac{1}{2\pi}(\phi_{GC}(t_k) \mod 2\pi)$$

where t_k is the time of kth occurrence of an R wave, and ϕ_{GC} is the instantaneous phase of GC. The time trace of $\Psi_{RR\text{-}GC}$ was visualized by plotting generalized relative phase versus time (Fig. 1).

As mentioned above, our THR was 150 beats·min^{-1}, and the locomotor rhythms, which were controlled at 2.5 Hz, were 150 steps·min^{-1} when running and 75 rpm when cycling. In addition, because we used EMG from only the right vastus lateralis muscle to estimate locomotor rhythms, the estimated rhythm was 1.25 (ζ) Hz (ζ is variability of locomotor rhythm). As a result, the 2:1 phase synchronization observed in the phase synchrogram corresponds to 1:1 entrainment of cardiac rhythm to locomotor rhythm as reported by Kirby et al. (1989a, 1989b, 1992) and Donville et al. (1993).

We used an index based on conditional probability to characterize synchronization strength (Stefanovska et al. 2000; Tass et al. 1998). To investigate the strength of the n:m phase synchronization between two oscillators, we regarded cardiac rhythm as a faster oscillator, $\phi_{RR}(t_k)$, and locomotor rhythm as a slower one, $\phi_{GC}(t_k)$. The interval of the faster oscillator ϕ_{RR}, $[0, 2\pi n]$, was divided into N bins. The values of $\phi_{GC}(t_j)$ belong to lth bin, and, in the case of $t_k - t_p/2 \le tj < t_k + t_p/2$, for each j the distribution is quantified as

$$r_l(t_k) = \frac{1}{M_l(t_k)} \sum_{i=1}^{M_l(t_k)} e^{i\phi_{GC}(t_j)}$$

According to Euler's formula,

$$r_l(t_k)^2 = \left(\frac{1}{M_l(t_k)} \sum_{i=1}^{M_l(t_k)} \cos\phi_{GC}(t_j)\right)^2 + \left(\frac{1}{M_l(t_k)} \sum_{i=1}^{M_l(t_k)} \sin\phi_{GC}(t_j)\right)^2$$

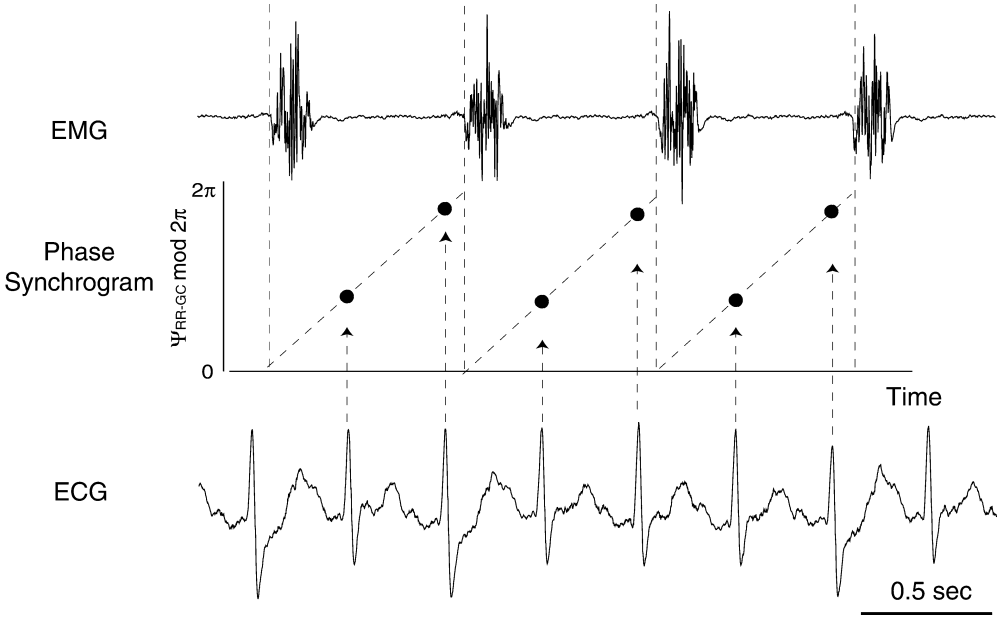

Fig. 1 The derivation of the cardio-locomotor synchrogram from the phase of locomotion and the time of the heart beats (R peaks in the ECG)

where $M_l(t_k)$ is the number of points in the bin at the kth instant and t_p is a sliding window period in which $t_p = 30$ s in this study. Where the phases were completely locked or completely unlocked we obtain $|r_l(t_k)| = 1$ or $|r_l(t_k)| = 0$, respectively.

Although an actual R wave occurs only at $\phi_{RR} \mathrm{mod} 2\pi = 0$, to improve reliability, we also calculated $|r_l(t_k)|$ over all bins and averaged them as follows:

$$\lambda_{n,m}(t_k) = \frac{1}{N}\sum_{l=1}^{N} |r_l(t_k)|$$

Since the purpose of our experiment was to measure the strength of the 2:1 phase synchronization between cardiac and locomotor rhythms when HR approached 150 beats·min^{-1} while running at 150 steps·min^{-1} and cycling at 75 rpm, we calculated $\lambda_{2,1}(\lambda_{2:1(CLS)})$.

In this study, we defined CLS as being present when $\lambda_{2:1(CLS)}$ exceeded 0.8, because when $\lambda_{2:1(CLS)}$ continuously exceeded 0.8, the phase relationship between cardiac and locomotor rhythms seemed to be a horizontal alignment. To quantify the characteristics of CLS, we first estimated the duration of CLS and the strength of CLS. The duration was defined as a period of continuous $\lambda_{2:1(CLS)} > 0.8$ throughout the 10- to 20-min measurement period. The strength was defined as mean $\lambda_{2:1(CLS)}$ in the period when $\lambda_{2:1(CLS)}$ exceeded 0.8 for the 10- to 20-min measurement period.

We used the surrogate data technique (Nomura et al. 2001) to determine whether the observed CLS occurred by chance or whether the two rhythms were linked. If two oscillators are independent, the phase relationship between them should be the same in surrogate data having the same statistical characteristics as the original data (Palus and Hoyer 1998). In this case, because the GC time series was stable throughout the measurement period, it was transformed to surrogate data, which was achieved by random shuffle, yielding a surrogate in which the mean, variation and histogram were the same as the original data.

Statistics

To evaluate HR level, mean GC, and mean duration of muscle contraction, the average of RRI, GC, and duration of muscle contractions during the 10- to 20-min measurement period were calculated for all subjects. Also, to compare variation of the locomotor rhythm during cycling and running, the standard deviation (SD) of the GC time series (SD_{GC}) under both exercise modes was calculated. For the CLS characteristics, CLS duration and mean $\lambda_{2:1(CLS)}$ under both exercise modes with the original and surrogate data were calculated. Data obtained for the characteristics of CLS during running and cycling tests and with the original and the surrogate data were compared using paired t tests. Values of $P < 0.05$ were considered significant.

Results

There were no significant differences in the mean values of RRI and GC between running and cycling subjects (Table 1), indicating that the intensity and locomotor rhythms were the same for both exercise types. Moreover, there was no significant difference between SD_{GC} for the two exercise modes, indicating no significant effect of exercise mode on the variability of locomotor rhythms. The mean values of RRI of both exercise modes during the 10- to 20-min measurement period correspond to about 151 beats·min^{-1}. There was no significant difference between exercise modes. Although

Table 1 RR interval, gait cycle and muscle contraction duration during running and cycling. Values are group means (SD). (*GC* gait cycle, *RR* RR interval, SD_{GC} standard deviation of GC time series)

Parameter	Run		Bicycle	
	Mean	(SD)	Mean	(SD)
RRI(ms)	396.6	(2.5)	395.0	(3.0)
GC				
Mean(ms)	799.9	(1.1)	800.2	(3.0)
SD_{GC} (ms)	18.1	(2.2)	17.7	(4.8)
Duration of muscle contraction (ms)	205.6	(20.2)	317.0*	(18.1)
Treadmill speed (m·min^{-1})	137.3	(48.4)	–	
Work rate (W)	–		131.8	(27.9)

*Significant difference from Run, $P < 0.05$.

THR was 150 beats·min^{-1}, mean HR during the 10- to 20-min measurement period was slightly above THR because there were subjects whose HR gradually increased near the end of the measurement period. However, the mean duration of right vastus lateralis muscle contraction was significantly greater when cycling than when running ($P < 0.05$).

Figure 2 shows representative RRI (Fig. 2A, A') and GC (Fig. 2B, B') time series, and the trace of $\Psi_{RR\text{-}GC}$ (Fig. 2C, C') recorded from one subject while he was running (Fig. 2A, B, C) and cycling (Fig. 2 A', B', C').

While the subject was running, three horizontal plateaus in $\Psi_{RR\text{-}GC}$ were observed after 9–15, 16–18, and 19–20 min of exercise, indicating the occurrence of CLS, which was about 0.4 and 0.9 of GC (Fig. 2C). GC was not specifically changed during CLS, but remained stable throughout the experimental period (Fig. 2B); likewise, HR variability remained steady during these periods (Fig. 2A).

On the other hand, as a consequence of differences in the phase relationship, $\Psi_{RR\text{-}GC}$, CLS occurred only intermittently during cycling and was not specific for any particular phase (Fig. 2C'). As the GC time series again remained steady throughout the experimental period (Fig. 2B'), we deduced that, in this case, CLS occurred when the cardiac and locomotor rhythms intersected during transitions of RRI (Fig. 2A').

We next used the surrogate data technique (Nomura et al. 2001) to determine whether the observed CLS resulted from entrainment of the cardiac rhythm by the locomotor rhythm, or whether it occurred when the cardiac and locomotor rhythms approached one another by chance. We found that, during running, the trace of the original $\Psi_{RR\text{-}GC}$ showed prolonged periods of CLS (Fig. 3A), whereas the surrogate data showed no horizontal plateaus in $\Psi_{RR\text{-}GC}$ together with repetitive phase drifts (Fig. 3B).

During cycling, by contrast, there was little difference between the original and surrogate data, and the trace of the original and surrogate $\Psi_{RR\text{-}GC}$ showed repetitive phase drifts (Fig. 4A, B).

In addition, as a result of changes in the strength of the CLS, $\Psi_{RR\text{-}GC}$ was significantly smaller after

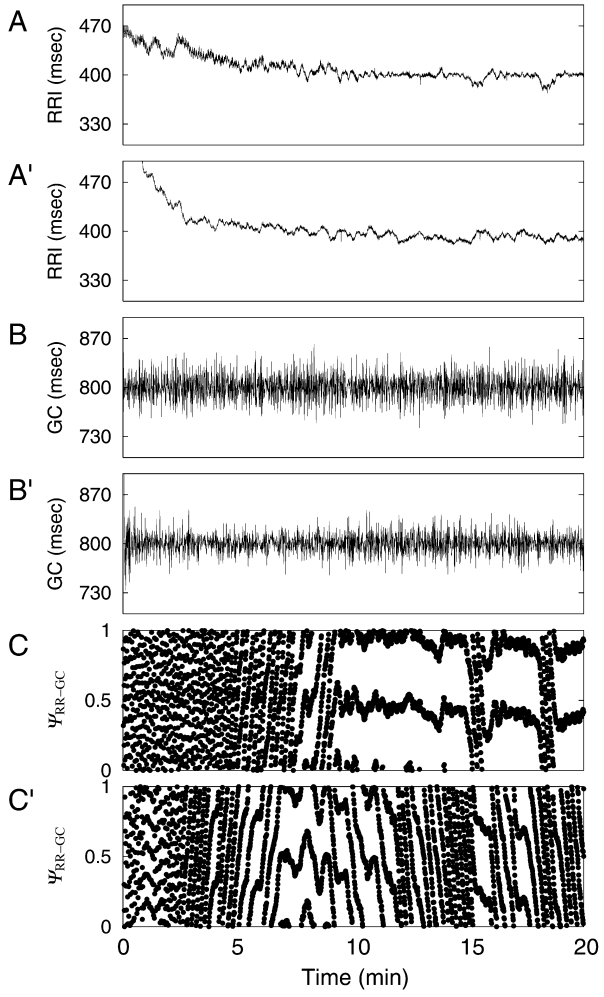

Fig. 2 Representative phase relationship between cardiac and locomotor rhythms in one subject. Shown are the time series of RR interval (*RRI*) (**A**, **A'**), gait cycle (*GC*) (**B**, **B'**) and the trace of the generalized relative phase difference between cardiac and locomotor rhythms (Ψ_{RR-GC}) (**C**, **C'**) during running (**A**, **B**, **C**) and cycling (**A'**, **B'**, **C'**). Note that whereas periods of cardiac and locomotor synchronization (*CLS*) are relatively prolonged and occur in certain phases during running, only brief periods of CLS occurred intermittently and in no specific phase during cycling

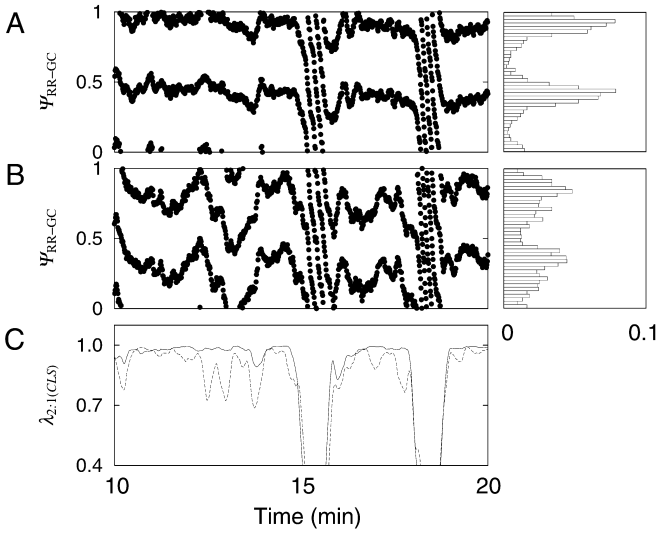

Fig. 3 Comparison of original and surrogate running data. Shown are the trace (*left side*) and the distribution (*right side*) of Ψ_{RR-GC} during the 10- to 20-min measurement period from a representative subject (data in Fig. 1 are from the same subject): **A** original data; **B** surrogate data; **C** time courses of $\lambda_{2:1(CLS)}$ for the original (*solid line*) and surrogate (*dashed line*) data. Note that after surrogation CLS was disrupted, and $\lambda_{2:1(CLS)}$ was diminished, especially after 12–14.5 min of exercise

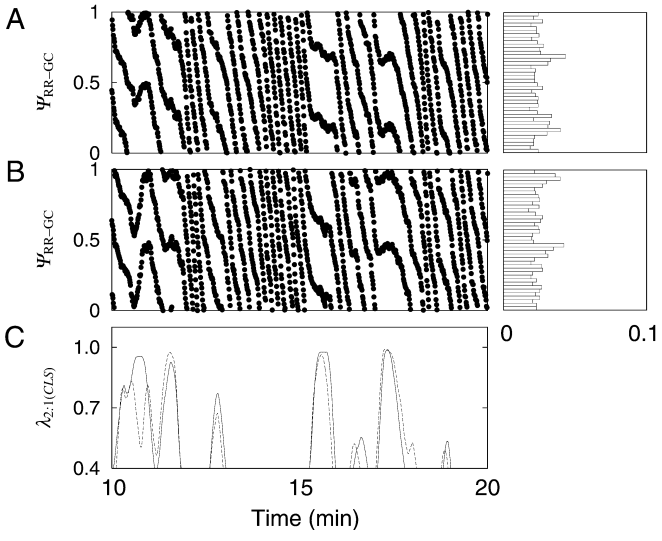

Fig. 4A–C Comparison of original and surrogate cycling data. Data from the same subject are presented as in Fig. 2. Note that surrogation had little effect on the data in this case

surrogation (Fig. 3C, solid line: original data; dashed line: surrogate data), particularly during the period spanning minutes 12–14 of the exercise. For the 10- to 20-min measurement period, the maximum CLS duration for the original and surrogate data were 294.5 s and 127.7 s, respectively. During cycling, by contrast, there were no specific differences in the maximum CLS duration for the 10–20 min of original and surrogate data, which were 28.6 s and 28.2 s, respectively.

For the distribution of Ψ_{RR-GC} in the 10- to 20-min measurement period during running, the histogram of the original Ψ_{RR-GC} showed two peaks at 0.3–0.4 and 0.8–0.9 (Fig. 3A, right). In contrast with the original data, the peaks in the histogram of Ψ_{RR-GC} of the surrogate data were lower (Fig. 3B, right). The distribution of Ψ_{RR-GC} in the 10- to 20-min measurement period during cycling revealed that there were no specific differences in the histograms of Ψ_{RR-GC} for the original and surrogate data: neither histogram had any peaks.

When the CLS criterion devised by Kirby et al. (1992) was adopted here, the difference between the original and surrogate Ψ_{RR-GC} trace during running observed by our method became vague (Fig. 5).

Also, the ratio between HR and gait rate, determined from 5-s data samples, showed that there was no

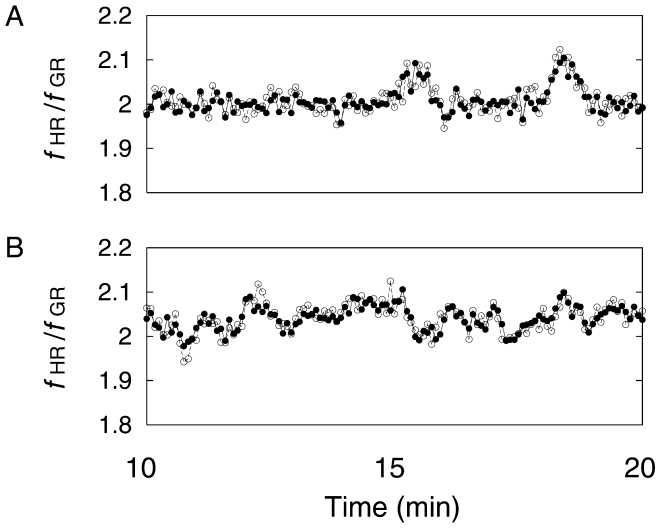

Fig. 5A, B CLS criterion of the frequency ratio of cardiac and locomotor rhythms. CLS was defined as being present when the frequency ratio of heart rate (*HR*) and gait rate (*GR*), which were determined as the average of 5-s data samples, became within 1.0% of each other. Shown are the time course of the frequency ratio between HR and GR for 5-s data samples every 5 s: **A** running; **B** cycling for the original (*solid line* and *solid circle*) and surrogate (*dashed line* and *open circle*) data. Note that surrogation had little effect on the data in exercise mode

difference in CLS between the original and surrogate data during running and cycling.

Finally, Table 2 shows that both the maximum durations of the CLS periods and the total CLS time were significantly ($P < 0.05$) longer during running than during cycling.

Furthermore, the mean values of $\lambda_{2:1(CLS)}$ in the measurement periods were significantly reduced by surrogation of the data obtained during running ($P < 0.05$), but there were no significant differences between the original and surrogate data obtained during cycling. Although there was no specific variation in the mean $\lambda_{2:1(CLS)}$ among subjects because the value was calculated in the period when $\lambda_{2:1(CLS)}$ was more than 0.8 for the 10- to 20-min measurement period, there was a little variation in the duration of CLS among subjects.

Table 2 Max duration and total duration of cardio-locomotor synchronization (CLS) and degree of CLS during running and cycling. Values are group means (SD). (*org*. the original data, *sur*. the surrogate data)

	Run		Bicycle	
	Mean	(SD)	Mean	(SD)
Max duration of CLS (s)	113.6	(66.5)	58.0*	(29.3)
Total duration of CLS (s)	368.5	(113.3)	137.2*	(73.0)
mean $\lambda_{2:1(CLS)}$				
org.	0.93	(0.02)	0.90*	(0.02)
sur.	0.92§	(0.04)	0.91	(0.05)

*Significant difference from Run, $P < 0.05$.
§Significant difference from the original data, $P < 0.05$.

Discussion

In the present study, we demonstrated that, during running, relatively long periods of phase synchronization between cardiac and locomotor rhythms occurred at specific phases using the phase synchrogram, and that the strength of the phase synchronization is diminished significantly after surrogation of the original data using the technique of Nomura et al. (2001) (Fig. 3). During cycling, by contrast, only comparatively brief, intermittent periods of phase synchronization occurred at no specific phase, and the strength of phase synchronization was changed little by surrogation (Fig. 4). This result, namely that the phase difference between cardiac and locomotor rhythms was not associated with a specific phase, agrees with the findings of Kirby et al. (1989a). It is highly likely that CLS occurring during running results from entrainment of the cardiac rhythm by the locomotor rhythm. During cycling, by contrast, the phase relationship seems to be due solely to CLS occurring when the cardiac rhythm approaches the locomotor rhythm by chance.

Methodological considerations

The rationale for using the phase synchrogram to investigate the phase relationship between cardiac and locomotor rhythms was manifested by Niizeki et al. (1993) and Udo et al. (1990), and was illustrated by the results of Kirby et al. (1989a). Niizeki et al. (1996) have shown that, during walking, the phase synchronization between cardiac and locomotor rhythms occurred at specific phases corresponding to 0.1–0.3 and 0.6–0.8 per interval of the left heel strikes. Our previous study (Nomura et al. 2001) has shown that, during running, the phase synchronization occurred at specific phases corresponding to 0.2–0.4 and 0.7–0.9 per GC. As shown in previous studies and the current study (Fig. 3A), during walking and running the phase synchronization between cardiac and locomotor rhythms at specific phases indicates that the timing of the muscle contractions corresponds to the end systolic to early diastolic phase of the cardiac cycle. By contrast, the results of Kirby et al. (1989a) showed that during cycling the phase difference between cardiac and locomotor rhythms gradually lengthened and shortened. This is consistent with our results (Fig. 4A).

The present study demonstrated that using the surrogate data technique (Nomura et al. 2001) allows us to reject the accidental provability of CLS during running, but not during cycling. Not all synchronization phenomena represent entrainment, since synchronization between two rhythms may occur by chance when one oscillator rhythm is close to that of another. To solve this problem, Kirby et al. (1991, 1992) used the cross-over test, in which the cardiac data from one subject are compared to the locomotor rate data from another.

Thus, CLS during hopping and skipping (Kirby et al. 1992) and finger tapping (Kirby et al. 1991) were shown not to be different statistically. Because they defined CLS as being when the cardiac and locomotor rates were within 1% of the closest integer ratio, phase differences between cardiac and locomotor rhythms were not considered. The frequency ratio between HR and gait rate, which were determined from brief (5 s) samples of data, showed no obvious differences in the integer ratio within 1.0% each other between the original and surrogate data during running and cycling (Fig. 5). These results indicate that beat-by-beat and/or step-by-step analysis of the relationship between cardiac and locomotor rhythms is needed to test whether CLS represents entrainment or occurs by chance.

Although past studies have qualitatively evaluated the degree of CLS according to its duration (Niizeki et al. 1993) or incidence (Kirby et al. 1989a, 1992), the current study has quantitatively demonstrated the duration and strength of CLS over 10–20 min using the $\lambda_{2:1(CLS)}$ index. In addition, using the surrogate data technique and the $\lambda_{2:1(CLS)}$ index, we determined the probability of CLS occurring by chance as a function of its duration. However, the $\lambda_{2:1(CLS)}$ index detects phase synchronization at any phase, not just specific ones. Therefore, the distribution of $\Psi_{RR\text{-}GC}$ is also required when the phase difference is important.

CLS originating directly from active muscles

Kirby et al. (1992) evaluated CLS in humans during skipping and hopping to investigate the relationship between the occurrence of CLS and vertical acceleration of the body. They suggested that the vertical movement of visceral tissue is unimportant to the CLS. When different modes of exercise are studied, such as running and cycling, there are differences in the vertical acceleration of the body and also in muscle contraction behavior, e.g., concentric versus eccentric. Our results show that, during cycling, the duration of muscle contraction is greater than during running (Table 1). Although differences in the characteristics of CLS between running (Fig. 3) and cycling (Fig. 4) cannot be explained solely from the results of the current study, one possible contributory factor is the difference in the duration of muscle contraction.

According to the concept of peripheral origin, CLS occurs because the afferent signal from active muscles during voluntary contraction is phase dependent within the cardiac cycle. Niizeki and Miyamoto (1999) speculated that the phase dependency of the afferent signal from muscle to the cardiovascular centers via group III fibers could modulate cardiac parasympathetic nervous (CPN) activity. In that regard, Niizeki and Miyamoto (1998, 1999) assessed the effects of the timing of muscle contraction within the cardiac cycle on the heart beat by estimating the phase response curve for muscle contractions within several cardiac phases. The phase response curve showed that muscle contractions occurring early in the cardiac cycle, i.e., during systole, provoke a shortening of RRI, whereas muscle contractions occurring in the middle or later phase of the cycle, i.e., during diastole, provoke a lengthening of RRI. Consequently, the slope of the phase response curve in the mid to later phase of the cardiac cycle is negative and therefore consistent with the condition of stable phase synchronization described in the theoretical analysis by Pavlidis (1973). Consistent with those findings, we observed that CLS during running occurs at 0.4 and 0.9 of GC (Fig. 3A), indicating that the timing of the muscle contractions corresponds to the end-systolic to early-diastolic phase of the cardiac cycle.

On the other hand, Niizeki and Miyamoto (1999) also reported that the phase response curve from some subjects did not exhibit the aforementioned characteristics. They speculated that if the duration of muscle contraction was comparable to the heart beat interval, even though the subjects were asked to contract their muscles impulsively as rapidly as possible, the phase-dependent heart beat response to the muscle contraction occurring within the cardiac cycle is smoothed or averaged over the duration of the muscle contraction. If this is the case it may explain why during cycling in the present study, CLS was intermittent and occurred at no specific phase; indeed, RRI was approximately 400 ms and the muscle contraction lasted 320 ms, which corresponds to 80% of the cardiac cycle.

If, as suggested above, CLS is the product of the phase-dependent signaling via group III muscle afferents to the cardiovascular centers, and that information in turn plays a role in inhibiting CPN activity (Niizeki and Miyamoto 1999), this inhibitory effect on CPN activity would likely induce a phase-dependent efferent signal and would be synchronized to the heart beat. Evidence supporting such a scenario includes the observations that: (1) stimulation of group III afferent fibers drives the cardiovascular centers (McMahon and McWilliam 1992; Rowell and O'Leary 1990); (2) with the muscle contracting every two heart beats, the frequency is too high to transmit an efferent signal via the sympathetic nervous system (Berger et al. 1989); (3) cardiac responses to sympathetic stimulation are phase-independent (Spear and Moore 1973); and (4) CPN activity is phase-dependent within the cardiac cycle (Levy et al. 1972; Yang et al. 1984). Recently, Nakamura et al. (1997) found that respiratory sinus arrhythmias (RSA) were decreased with muscle contraction during inspiration and increased with muscle contractions during expiration. By way of explanation, they suggested that afferent information from contracting muscles might inhibit the CPN activity, which is consistent with the concept of Niizeki and Miyamoto (1999).

CLS of indirect peripheral origin

One alternative explanation for the difference of CLS characteristics could be that locomotor-induced changes in thoracic and abdominal pressures vary the rate of

venous return (VR) and/or ventricular ejection (VE), and thereby influence cardiac rhythm (Simmons et al. 1997). Simmons et al. (1997) observed intermittent CLS in trotting dogs, and suggested that the variation in cardiac period and the resulting intermittent CLS are a function of locomotor and ventilatory influence on VR and/or VE. They emphasized that systole began early in the support phase of locomotion during the intermittent CLS. In running mammals, the thorax is subjected to repeated impact loading as the limbs strike the ground (Bramble and Carrier 1983). However, there would be little impact loading in cycling humans. Although, locomotor-induced changes in thoracic and abdominal pressures may vary the rate of VR and/or VE in four-legged species (Simmons et al. 1997) and running humans, locomotor-induced changes are expected to be less pronounced in cycling humans.

Another alternative possibility is that respiratory rhythms exert an effect on cardiac rhythm. If so, CRS would likely be indicative of CLS when LRS occurs during rhythmic exercise. This indirect peripheral origin hypothesis may explain the difference in the characteristics of CLS seen with running and cycling because, like CLS, LRS occurs intermittently during cycling but for more prolonged periods during running (Paterson et al. 1986, Bernasconi and Kohl 1993). In addition, CRS occurs while individuals are at rest (Kenner et al. 1976; Schäfer et al. 1998; Seidel and Herzel 1998; Galletly and Larsen 1990). Niizeki et al. (1993) reported that both CLS and LRS occur during walking, when respiratory and locomotor rhythms are spontaneous; however, LRS was abolished when subjects voluntarily synchronized locomotor rhythm to cardiac rhythm. They speculated that to exhibit CLS, LRS might need to be abolished, but emphasized that this was mere speculation. Since the effects of respiratory rhythm on CLS cannot be determined from the results of the current study, further study would be needed to test these effects.

CLS of central origin

The cardiac rhythm is modulated by the centrally generated locomotor rhythm. For example, Kawahara et al. (1993, 1994) reported that heart beat fluctuations are modulated by the frequency of locomotor rhythm, which is indicated by integrated efferent discharges (time constant 0.1 s) in the hind-limb muscle nerve during fictive locomotion in decerebrate cats whose hind-limb muscle nerves have been dissected peripherally to eliminate afferent discharge from the muscles. However, although they found a coherence peak in heart beat fluctuations at a frequency corresponding to the mean stepping frequency, they did not evaluate the phase relationship between cardiac and locomotor rhythms. Their findings nonetheless suggest that the modulation of the heart beat rhythm by locomotor rhythm originates centrally, and is the product of a direct interaction between the cardiovascular centers and the central pattern generator, though it is not clear whether CLS actually occurred. If CLS is originated by neural circuits in the central nervous system, the characteristics of CLS observed during different exercise modes that have the same locomotor rhythm and the same intensity may resemble each other.

To address that question, we compared CLS during cycling and running using a protocol in which the two exercise modes had the same locomotor rhythm and the same intensity. In addition, we used the surrogate data technique to assess whether the observed CLS resulted from entrainment of cardiac to locomotor rhythms or from chance. Our results show that the characteristics of the observed CLS during running and cycling were different (Figs. 3, 4; Table 2). Therefore, the central origin hypothesis might not be able to account for the CLS that occurs in rhythmically exercising humans. The locomotor central pattern generator, however, might behave differently according to the exercise mode undertaken. In this study, the duration of muscle contraction differed between running and cycling.

In conclusion, the present study showed differences between running and cycling in the characteristics of CLS. These differences may be used to explain the observed difference in the duration of muscle contraction between the two modes of locomotion. For running, the phase-dependent afferent signals from the muscle might influence cardiovascular centers and inhibit CPN activity when a relative short duration of muscle contraction occurs within one cardiac cycle. By contrast, for cycling, because the duration of muscle contraction is long enough within one cardiac cycle, the phase-dependent effect of afferent signals from the muscle might be smoothed or averaged. Further studies are needed to investigate the phase-dependent heart beat response by muscle contractions during running.

Acknowledgements We are grateful to Professor Y. Yamamoto (Graduate School of Education, The University of Tokyo, Tokyo, Japan) for his useful suggestions, and to Professors M. Yamaguchi and H. Ando (Faculty of Health Science, Kobe University School of Medicine, Kobe, Japan) for their valuable discussions.

References

Bechbache RR, Duffin J (1977) The entrainment of breathing frequency by exercise rhythm. J Physiol (Lond) 272:553–561

Berger RD, Saul JP, Cohen RJ (1989) Transfer function analysis autonomic regulation I. Canine atrial rate response. Am J Physiol 256:H142–H152

Bernasconi P, Kohl J (1993) Analysis of co-ordination between breathing and exercise rhythms in man. J Physiol (Lond) 471:693–706

Bramble DM, Carrier DR (1983) Running and breathing in mammals. Science 219:252–256

Donville JE, Kirby RL, Doherty TJ, Gupta SK, Eastwood BJ, MacLeod DA (1993) Effect of cardiac-locomotor coupling on the metabolic efficiency of pedalling. Can J Appl Physiol 18:379–391

Galletly DC, Larsen PD (1990) The determination of cardioventilatory coupling from heart rate and ventilatory time series. Res Exp Med Berlin 199:95–99

Grillner S (1975) Locomotion in vertebrates: central mechanisms and reflex interaction. Physiol Rev 55:247–304
Kawahara K, Yoshioka T, Yamauchi Y, Niizeki K (1993) Heart beat fluctuation during fictive locomotion in decerebrate cats: locomotor-cardiac coupling of central origin. Neurosci Lett 150:200–202
Kawahara K, Yamauchi Y, Niizeki K, Yoshioka T (1994) Interactions between respiratory, cardiac and stepping rhythms in decerebrated cats: functional hierarchical structures of biological oscillators. Method Inform Med 33:129–132
Kenner T, Pessenhofer H, Schwaberger G (1976) Method for the analysis of the entrainment between heart rate and ventilation rate. Pflugers Arch 363:263–265
Kirby RL, MacLeod DA, Marble AE (1989a) Coupling between cardiac and locomotor rhythms: the phase lag between heart beats and pedal thrusts. Angiology 40:620–625
Kirby RL, Nugent ST, Marlow RW, MacLeod DA, Marble AE (1989b) Coupling of cardiac and locomotor rhythms. J Appl Physiol 66:323–329
Kirby RL, Carr SE, MacLeod DA (1990) Cardiac-locomotor coupling while finger tapping. Percept Motor Skills 71:1099–1104
Kirby RL, Gupta SK, Carr SE, MacLeod DA (1991) Cardiac-locomotor coupling while finger tapping: Part II. A cross-over control study. Percept Motor Skills 73:831–834
Kirby RL, Atkinson SM, Donville JE, Urdang MF, Stanley DA, Gupta SK, MacLeod DA (1992) Failure of accentuated vertical body movements to induce cardiac-locomotor coupling. J Appl Physiol 72:1368–1374
Levy MN, Iano T, Zieske H (1972) Effects of repetitive bursts of vagal activity on heart rate. Circ Res 30:186–195
McMahon SE, McWilliam PN (1992) Changes in R-R interval at the start of muscle contraction in the decerebrate cat. J Physiol (Lond) 447:549–562
Nakamura Y, Hayashi N, Muraoka I (1997) Temporal effect of muscle contraction on respiratory sinus arrhythmia. Method Inform Med 36:268–270
Niizeki K, Miyamoto Y (1998) Cardiolocomotor interactions during dynamic handgrip and knee extension exercises. Phase-locked synchronization and its physiological implications. Adv Exp Med Biol 450:199–206
Niizeki K, Miyamoto Y (1999) Phase-dependent heartbeat modulation by muscle contractions during dynamic handgrip in humans. Am J Physiol 276:H1331–H1338
Niizeki K, Kawahara K, Miyamoto Y (1993) Interaction among cardiac, respiratory, and locomotor rhythms during cardiolocomotor synchronization. J Appl Physiol 75:1815–1821
Niizeki K, Kawahara K, Miyamoto Y (1996) Cardiac, respiratory, and locomotor coordination during walking in humans. Folia Primatol Basel 66:226–239
Nomura K, Takei Y, Yanagida Y (2001) Analysing entrainment of cardiac and locomotor rhythms in humans using the surrogate data technique. Eur J Appl Physiol 84:373–378
Palus M, Hoyer D (1998) Detecting nonlinearity and phase synchronization with surrogate data. IEEE Eng Med Biol Mag 17:40–45
Paterson DJ, Wood GA, Morton AR, Henstridge JD (1986) The entrainment of ventilation frequency to exercise rhythm. Eur J Appl Physiol 55:530–537
Pavlidis T (1973) Biological oscillators: their mathematical analysis. Academic Press, New York
Rosenblum MG, Kurths J, Pikovsky A, Schäfer C, Tass P, Abel HH (1998) Synchronization in noisy systems and cardiorespiratory interaction. IEEE Eng Med Biol Mag 17:46–53
Rowell LB, O'Leary DS (1990) Reflex control of the circulation during exercise: chemoreflex and mechanoreflexes. J Appl Physiol 69:407–418
Schäfer C, Rosenblum MG, Kurths J, Abel HH (1998) Heartbeat synchronized with ventilation. Nature 392:239–240
Schäfer C, Rosenblum MG, Abel H, Kurths J (1999) Synchronization in the human cardiorespiratory system. Physiol Rev E 60:857–870
Seidel H, Herzel H (1998) Analyzing entrainment of heartbeat and respiration with surrogates. IEEE Eng Med Biol Mag 17:54–57
Simmons AD, Carrier DR, Farmer CG, Gregersen CS (1997) Lack of locomotor-cardiac coupling in trotting dogs. Am J Physiol 273:R1352–R1360
Spear JF, Moore EN (1973) Influence of brief vagal and stellate nerve stimulation on pacemaker activity and conduction within the atrioventricular conduction system of the dog. Circ Res 32:27–41
Stefanovska A, Haken H, McClintock PVE, Bajrović MHF, Ribarič S (2000) Reversible transitions between synchronization states of the cardiorespiratory system. Physiol Rev Lett 85:4831–4834
Tass P, Rosenblum MG, Weule J, Kurths J, Pikovsky A, Volkmann J, Schnitzler A, Freund HJ (1998) Detection of n:m phase locking from noisy data: application to magnetoencephalography. Physiol Rev Lett 81:3291–3294
Udo M, Yoshida T, Nakazumi T, Kinugawa K, Watanabe A (1990) Occurrence of entrainment of heart beat into endurance running pitch and its significance in energy consumption. In Kaneko M (ed) Fitness for the aged, disabled, and industrial worker. Volume 20 of International Series of Sport Science. Human Kinetics Books, Champaign, Ill., pp 79–83
Yang T, Jacobstein MD, Levy MN (1984) Synchronization of automatic cells in S-A node during vagal stimulation in dogs. Am J Physiol 246:H585–H591