Nonlinear dynamics of the frequency locking of baroreceptor and sympathetic rhythms

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Gebber, Gerard L., Sheng Zhong, Shi-Yi Zhou, and Susan M. Barman. Nonlinear dynamics of the frequency locking of baroreceptor and sympathetic rhythms. Am. J. Physiol. 273 (Regulatory Integrative Comp. Physiol. 42): R1932–R1945, 1997.—We used phase plane analysis to identify modes of frequency locking of the 10-Hz rhythm in sympathetic nerve discharge (SND) to the cardiac cycle in urethane-anesthetized, baroreceptor-inervated cats. Frequency locking occurred in rational ratios predicted by a generic mathematical construct called the Farey tree. Both simple harmonic ratios (e.g., 1:3) and complex ratios (e.g., 2:5) comprised of relatively prime integers (no common divisor) were identified under natural conditions. Frequency locking in such ratios is attributed to forcing of the 10-Hz oscillator by pulse-synchronous baroreceptor afferent nerve activity (BNA). Ventricular pacing changed the frequency of the 10-Hz rhythm as well as heart rate so as to maintain or change the ratio of frequency locking in a predictable way. Intriguingly, frequency locking of the 10-Hz rhythm to medullary raphe sympathoinhibitory stimuli in simple harmonic ratios was accompanied by increased power in the 10-Hz band of SND, whereas locking in complex ratios led to decreased 10-Hz power. These findings raise the possibility that pulse-synchronous BNA also exerts divergent actions on the 10-Hz rhythm depending on the ratio of frequency locking. Augmented 10-Hz power can be attributed to the resonant properties of oscillators that are periodically forced at the same phase in their cycle.

bispectral analysis; Farey series; linear coherence; phase plane analysis; synchrony

Normally, biological oscillators are not free running. Rather, they are coupled to other oscillators and/or entrained by periodic inputs from the external and internal environments. Periodic perturbation of an oscillator changes the amplitude, frequency, and phase of its output. Characterization of the changes in these parameters not only provides information concerning the intrinsic properties of the oscillator but may also help to explain physiological processes such as interlimb coordination (7, 26, 28, 31, 32), respiratory-locomotor coupling (11), interaction of normal and abnormal cardiac pacemakers leading to complex arrhythmias (23), and the control of sympathetic nerve discharge (SND) by pulse-synchronous baroreceptor afferent nerve activity (BNA). BNA-SND interactions are the subject of this paper.

SND in the cat is heterogeneous in terms of its frequency components, a fact that is often ignored when considering baroreceptor control mechanisms. Two of these components, the cardiac-related and 10-Hz rhythms, are intermixed in variable proportions in baroreceptor-inervated cats and comprise a significant proportion of the total power in SND (3, 4). Work from this laboratory (19, 20) supports the view that frequency locking of centrally generated 2- to 6-Hz oscillations in a 1:1 relationship to pulse-synchronous BNA accounts for the cardiac-related rhythm in SND. The interactions between pulse-synchronous BNA and the 10-Hz rhythm are more complex and incompletely characterized. Because heart rate in the anesthetized cat is near 3 beats/s and the frequency of the 10-Hz rhythm is in the range of 7–13 Hz (4, 22), 1:1 locking of the 10-Hz rhythm in SND to pulse-synchronous BNA is unlikely to occur. However, instances of 1:3 locking of the cardiac cycle and the 10-Hz rhythm have been reported (4, 13, 22, 30). Thus the two rhythms can become nonlinearly coupled.

Nonlinear frequency locking of oscillators occurs in rational ratios of integers (10, 28). In cases when the higher frequency is a multiple of the lower, we refer to frequency locking in terms of a simple harmonic ratio (e.g., 1:3, 1:4). Nonlinear frequency locking of oscillators in ratios comprised of relatively prime integers (no common divisor; e.g., 2:5, 3:10) is also possible. We refer to these as complex ratios. All possible ratios of frequency locking (all rational numbers between 0 and 1) can be ordered into a hierarchy called the Farey tree (1, 15, 23, 27). To grow the tree, the numerators and denominators of adjacent ratios (starting with the parents 0:1 and 1:1 in level 0) are added together according to the following formula

\[ \frac{p}{q} + \frac{p'}{q'} = \frac{p + p'}{q + q'} \]

The ratios in levels 0–4 of the Farey tree and some of the ratios in level 5 are shown in Fig. 1. Note that the lowest-order complex ratio (2:3) does not appear until level 2 of the tree.

Numerical studies of coupled or forced oscillators have provided important information on the relative stability of frequency locking in different ratios (1, 15, 23, 27, 28). The stability of frequency locking for each ratio is reflected by the width of its Arnold’s tongue, named after the Russian mathematician who first described them. Each tongue represents a region of parameter space corresponding to pure frequency locking in a particular rational ratio. The parameters that make up the space are the ratio of the frequencies, the strength of coupling of the components, and a measure of the relative stability of the intrinsic phase states, \( \phi = 0 \) and \( \phi = \pi \) (28). Low-order ratios have wider tongues than high-order ratios, and thus are more structurally stable. As a general rule, the stability of frequency locking is inversely related to the denominator of the ratio (28).
Because frequency locking of the cardiac cycle and the 10-Hz rhythm in SND can occur in low-order simple harmonic ratios of 1:3 and 1:4, coupling in the higher-order complex ratio of 2:7 is predicted by the Farey tree (Fig. 1). However, whether stable frequency locking in this and even higher-order ratios can be attained in a noisy biological system is unclear. This is because of the relative instability (narrow Arno’d tongues) of frequency locking in high-order ratios. As a consequence, perturbations are more apt to disrupt high-order than low-order frequency locking. Regarding this point, the first objective of the current study was to identify those simple harmonic and complex ratios at which stable frequency locking of pulse-synchronous BNA and the 10-Hz rhythm in SND is possible. A second objective was to compare the functional consequences (as reflected by changes in 10-Hz power) of frequency locking in simple harmonic versus complex ratios. Finally, we dealt with the question of whether the central circuits responsible for the 10-Hz rhythmic discharges of sympathetic nerves with different targets are independently controlled by pulse-synchronous BNA.

METHODS

Experimental subjects and anesthesia. The data analyzed were available on magnetic tape from 44 cats, 22 of which had been used in previous investigations (4, 22). The protocols used were approved by the All-University Committee on Animal Use and Care of Michigan State University. After initial induction with isoflurane (2.5% mixed with 100% O2), spontaneous respiration after anesthesia was eunepic with end-tidal CO2 (Traverse Medical Monitors Capnometer model 2200) in the normocapnic range. Subsequently, the animal was paralyzed (galamine triethiodide, 4 mg/kg iv, initial dose), pneumo-traumatized, and artificially ventilated with room air enriched with 100% O2. End-tidal CO2 was kept near 4% by adjusting the rate and volume of artificial ventilation. Rectal temperature was kept near 38°C with a heat lamp. In experiments with medullary raphe stimulation, baroreceptor denervation was performed by bilateral section of the carotid sinus, aortic depressor, and vagus nerves in the neck. Complete baroreceptor denervation was indicated by abolition of coherence of SND to the arterial pulse at the frequency of the heart beat (i.e., elimination of cardiac-related rhythm).

A Grass S8800 quartz-timed digital stimulator and PSIU6 constant-current unit were used to deliver 1-ms square-wave pulses of variable intensity (20–200 µA) through concentric bipolar stainless steel electrodes (Rhodes model SNE-100 with 0.25-mm tip exposures separated by 0.75 mm) to sympathoinhibitory sites in the caudal medullary raphe nuclei. Interpulse intervals were accurate to within ±0.05%. The stimulating electrodes were positioned in the medullary raphe complex after removal of portions of the occipital bone and medial cerebellum. The sites of raphe stimulation were on the midline, 2–3 mm rostral to the obex and 3–5 mm below the dorsal surface of the medulla. High-frequency (50 Hz) stimulation of these raphe sites completely inhibited SND, whereas single shocks induced a brief reduction in SND lasting 50 ms (onset latency, 70 ms) that was followed by resetting of the 10-Hz rhythm (see Fig. 2 in Ref. 24).

The heart was paced electrically in 11 baroreceptor-innervated cats. Pulses (500–800 µA, 1-ms duration) were applied though a pair of platinum electrodes inserted into the wall of the left ventricle. Ventricular rate was increased by setting the frequency of stimulation above the control heart rate. Ventricular rate was decreased below control by using paired pulses separated by 160–220 ms applied at the desired frequency (see Ref. 22 for details).

Mental recordings. Potentials were recorded monophasically with bipolar platinum electrodes from the central ends of the cut left inferior cardiac and left renal postganglionic sympathetic nerves. The nerves were isolated and prepared as previously described (4, 21). SND was monitored initially by using a preamplifier band pass of 1–1,000 Hz so that bursts of multunit spikes appeared as slow waves (4, 13, 20). The frontal-parietal EEG was recorded with a gold-plated disc electrode placed on the skull and the indifferent electrode on crushed muscle; the preamplifier band pass was 1–1,000 Hz.

Data analysis. The linear and nonlinear methods used have been described in detail in earlier reports from this laboratory (4, 21, 22, 24, 29). Because of the involved nature of the analysis in this study, a flow chart depicting the complete process is presented in Fig. 2. SND was low-pass filtered at 50 Hz and sampled at 100 Hz (Fig. 2A, bottom). This signal is referred to as the original record. The same sampling rate was used for the arterial pulse (Fig. 2A, top). Autospectra of the arterial pulse and the original record of SND and coherence functions measuring the strength of linear correlation (scale 0–1.0) of these signals were computed by using a modified version (29) of the program of Cohen et al. (14). Fast Fourier transform (FFT) was usually performed on 32 10-s windows with 50% overlap (160-s data block). The resolution of measurement was 0.1 Hz/bin, and...
the spectra were displayed in most cases for frequencies between 0 and 20 Hz. The frequency values cited in the text were obtained from digital readouts. Typically, the autospectrum of inferior cardiac SND contained two large peaks, the first at the frequency of the heartbeat and the second in the 10-Hz band. The ratio of the amplitudes of the 10-Hz to the cardiac-related peaks in 74 autospectra averaged 0.75 ± 0.12.

In the autospectrum of SND shown in Fig. 2B, middle, the first large peak was near 3 Hz and the second large peak coincided with the fourth harmonic of the heart rate. The high coherence value (>0.9) relating SND to the arterial pulse at the frequency of the heartbeat (Fig. 2B, bottom) atest to the presence of a strong cardiac-related rhythm in SND. A coherence value of ≥0.1 reflects a significant linear relationship between two signals when 32 windows are averaged (8). The higher-frequency component in SND was strongly correlated (coherence value 0.88) to the fourth harmonic of the arterial pulse. This relationship indirectly suggests 1:4 frequency locking (coherence value 0.88) to the fourth harmonic of the heart rate in the autospectrum of the original signal. The band-pass filtered record of the arterial pulse (Fig. 2B, top) likely reflect the nonsinusoidal shape of the pulse (22).

Frequency locking can be detected directly by plotting the forcing signal (pulse-synchronous BNA as reflected by the arterial pulse) against the forced signal (SND) on the x and y coordinates of a plane. Such plots are called Lissajous diagrams or phase plane plots (9, 33). They contain loops whose form is dependent primarily on three factors: the ratio of frequency locking, the phase between the two signals, and their relative amplitudes. Frequency locking exists when successive loops are similar in form and closely superposed. In contrast, no clear pattern appears when frequency locking is absent, as is the case for a quasiperiodic relationship with an irrational ratio (10, 28). Rather, the plane quickly fills in due to the sliding relationships between the signals.

Lissajous diagrams were constructed in the following way. Digital band-pass filtering without phase distortion was performed with software from RC Electronics, Santa Barbara, CA. This allowed us to isolate the frequency components of interest and minimize the influence of other components of the signal on the shape of the loops. Within the designated band pass, the original power was reduced by no more than 24%. The roll-off efficiency (slope) of the filter was such that power outside of the band pass was further reduced by 39%/Hz. For the arterial pulse, the width and center frequency of the band pass are set to match that of the peak at the frequency of the heartbeat in the autospectrum of the original signal. The band-pass filtered record of the arterial pulse is sinusoidal in shape and nearly constant in amplitude (Fig. 2C, top). For SND, the original record is processed to give two digitally filtered signals, one (Fig. 2C, middle) with the same band pass used for the arterial pulse and the other (Fig. 2C, bottom) with a band pass whose width and center frequency are those of the peak near 10 Hz in the autospectrum of SND. Note that the band pass-filtered records of SND are sinusoidal in shape although there is more amplitude modulation than in the band pass-filtered record of the
arterial pulse. After digital filtering, pairs of signals are plotted against each other at 10-ms intervals. Lissajous diagrams are constructed from 16 consecutive 5-s data segments to assess the stability of frequency locking. The program for phase plane analysis (also from RC Electronics) determines the minimum and maximum amplitudes of each signal and uses them to set the scale of the x- and y-axes of the Lissajous diagram. One-to-one relationships are reflected by elliptical orbits whose shape is dependent on the phase angle between the signals and their relative amplitudes (9, 33). The closely superposed elliptical orbits in Fig. 2D, left, attest to 1:1 locking of the low-frequency component of SND to the arterial pulse. Phase angle is not considered in this study.

The loops relating the arterial pulse (Fig. 2D, middle) or cardiac-related sympathetic nerve slow wave (Fig. 2D, right) to the 10-Hz oscillation in SND are more complicated. When these diagrams are followed from left to right, it can be noted that two cycles of the 10-Hz rhythm were completed for each one-half cycle of the lower-frequency rhythm. The trace then closely repeats itself, moving in the reverse direction, thereby completing one loop. The close superposition of many such loops provides direct evidence for frequency locking in a simple harmonic ratio of 1:4. As a rule, the ratio of frequency locking is simply the number of complete oscillations in the two directions (x, y) during the production of a complete loop (9). The loops in the Lissajous diagram relating the arterial pulse to the 10-Hz sympathetic nerve slow wave (Fig. 2D, middle) were more closely superposed than those in the diagram relating the cardiac-related and 10-Hz slow waves in SND (Fig. 2D, right). A factor contributing to the difference is the relative constancy of the amplitude of the filtered arterial pulse (Fig. 2C, top) compared with that of the cardiac-related sympathetic nerveslow wave (Fig. 2C, middle).

A second factor more difficult to assess might involve differences in the strengths of phase locking of these pairs of signals. Because the amplitude of the filtered arterial pulse always was more stable than that of the cardiac-related sympathetic nerve slow wave and because the arterial pulse is presumed to reflect more accurately pulse-synchronous BNA, this signal is plotted against the 10-Hz sympathetic nerve slow wave in the Lissajous diagrams of Figs. 4, 5, 7, 8, and 10–13. Standardized 1-ms electrical square-wave pulses applied to medullary raphe sites were also used to construct Lissajous diagrams (see RESULTS).

Nonlinear interactions of the cardiac-related and 10-Hz rhythmic discharges of the same sympathetic nerve were also identified by using bispectral analysis. Whereas the autospectrum is the FFT of the first-order autocovariance, the bispectrum and its normalized version (bicoherence function) are the spectral counterparts of the second-order autocovariance (17, 25). As such, bispectral analysis identifies relationships among frequency triples in the same signal where the third frequency is the sum of the other two due to quadratic nonlinear phase locking.

The bicoherence function of SND was computed by using a modified version (22) of the program of Dumermuth and Gasser (16) based on the formulas in Refs. 17 and 25. The data segments (32 10-s windows with 50% overlap) analyzed were the same as those used to calculate the autospectrum. The bicoherence values for the data are compared with idealized values for predefined probability levels under a Gaussian hypothesis (see SUBROUTINE BICOPH on p. 237–238 in Ref. 16). The comparison allows one to decide whether the frequency components of the signal are statistically independent (i.e., linearly superposed) or nonlinearly interactive (see details in Ref. 22). A P value of $10^{-4}$ is used as the threshold for significant phase locking of a frequency triple. This threshold reflects the 95% confidence limits of the bispectrum (also calculated by the program), which serves as a second measure of the strength of phase locking (5, 17). The absolute value of the bispectrum is a measure of the shifting of the origin of the higher frequency (e.g., 10-Hz rhythm) with respect to the lower frequency (e.g., cardiac-related rhythm). The 95% confidence limits of the bispectrum were less than or equal to $\pm 20^\circ$ for a P value of $10^{-3}$. By comparison, the 95% confidence limits were much wider (greater than or equal to $\pm 130^\circ$) for P $\geq 10^{-3}$.

The bicoherence function in Fig. 2E should be read as follows. Frequencies $f_1$ and $f_2$ are plotted symmetrically as x and y coordinates. The third frequency in each phase-locked triple is the sum of $f_1$ and $f_2$. The P value reflecting the strength of phase locking of a frequency triple is provided by digital readout and represented by the pattern of black dots on the white background (see scale in Fig. 2E). The significant bicoherence levels (P $\leq 10^{-3}$) plotted near x,y coordinates 3 and 12 Hz reflect phase locking of the cardiac-related and 10-Hz rhythms and their sum, a modulated frequency. The significant bicoherence levels (P $\leq 10^{-3}$) plotted near x,y coordinates 3 and 9 Hz reflect phase locking of the cardiac-related rhythm, the difference between the 10-Hz and cardiac-related rhythms (also a modulated frequency) and their sum, the 10-Hz rhythm. The two regions of significant bicoherence on the diagonal line (line of equal frequency) at x,y coordinates near 3 and 3 Hz and 12 and 12 Hz reflect phase locking of the cardiac-related and 10-Hz rhythms to their respective second harmonics. These regions indicate that the cardiac-related and 10-Hz slow waves were not pure sinusoids (22).

Values in the text are expressed as means $\pm$ SE.

RESULTS

Frequency locking in a complex ratio. Figure 3 shows the autospectra of the arterial pulse (Fig. 3, top) and
inferior cardiac SND (Fig. 3, middle) and the corresponding coherence functions (Fig. 3, bottom) from two experiments (A, B) in which the 10-Hz rhythm was not a multiple (harmonic) of the heart rate. Note that the coherence values relating SND to the arterial pulse approached 1.0 at the frequency of the heart beat. The coherence values were also statistically different from zero at some of the harmonics of the heart rate despite the low power in SND at these frequencies. In contrast, the 10-Hz rhythm in SND was not linearly correlated to the arterial pulse (zero coherence). In each of these experiments, the frequency of the 10-Hz rhythm fell between the second and third harmonics of the heart rate.

The Lissajous diagrams from the same experiments are shown in Fig. 4. The diagrams were derived from digitally filtered records (5-s strip; Fig. 4, top) of the arterial pulse and SND (10-Hz band). In Fig. 4A (same experiment as in Fig. 3A), the arterial pulse and the 10-Hz slow wave in SND were locked in a complex ratio of 2:5. This is indicated by the closely superposed loops in the Lissajous diagram that form a figure 8 with a "tail" placed on its side. Following the diagram from top right to bottom right, note that 2:5 sympathetic nerve slow waves are completed during each arterial pulse. The trace then closely repeats itself, moving from bottom right to top right, thereby completing one loop showing 2:5 frequency locking. The portion of the 5-s data strip between the arrows (see Fig. 4, top traces) was expanded and placed below the Lissajous diagram. The dotted lines in the expanded digitally filtered records also show the 2:5 relationship between the arterial pulse and the 10-Hz rhythm in SND. Note that the peak of every other arterial pulse coincided with that of every fifth 10-Hz slow wave.

In contrast, no clear pattern appeared in the Lissajous diagram in Fig. 4B (same experiment as in Fig. 3B). Rather, each loop is shifted in position relative to the one preceding it. As a consequence, the diagram fills in with near uniform density within the 5-s period of analysis. In this experiment, the ratio of the heart rate to the 10-Hz rhythm in SND was closest to 5:12. As expected, however, the dotted lines in the expanded records below the Lissajous diagram failed to show strict phase locking of the arterial pulse and the 10-Hz sympathetic nerve slow wave in this ratio.

As a test of the sensitivity of phase plane analysis in detecting frequency locking within 5-s data segments, we constructed Lissajous diagrams relating standardized 1-V sine waves reflecting pulses generated independently by two channels of a quartz-timed Grass S8800 digital stimulator. Figure 5A shows the Lissajous diagram for sine waves with frequencies of 3.0 and 10.0 Hz. Although complex, the pattern in the diagram is repeated almost exactly five times during the 5-s period of analysis, which included 50 cycles of the higher frequency and 15 cycles of the lower frequency. Changing the higher frequency from 10.0 to 10.1 Hz obliterated the pattern in the Lissajous diagram (Fig. 5B). Such tests demonstrate that changes in frequency equal to the resolution of measurement in the autospectra (0.1 Hz/bin) dramatically alter the pattern and degree of superposition of loops in the Lissajous diagram.

Although the Lissajous diagram in Fig. 4B showed no sign of frequency locking of the 10-Hz rhythm to the cardiac cycle, the bicoherence function of inferior cardiac SND in this experiment (Fig. 6B) showed phase locking of the cardiac-related and 10-Hz rhythms and their modulated frequencies (sum and difference). Phase locking of such frequency triples occurred in 12 of 16 cases when the Lissajous diagram showed no clear pattern of frequency locking. In contrast, frequency locking of the cardiac cycle and the 10-Hz rhythm in simple harmonic or complex rational ratios (total of 58 cases) always was accompanied by significant phase locking of frequency triples in SND made up of the cardiac-related and 10-Hz rhythms and their modu-
lated frequencies. The bicoherence function in Fig. 6A corresponds to the Lissajous diagram in Fig. 4A.

Ratios of frequency locking. The Lissajous diagrams in Figs. 7 and 8 show examples of frequency locking of the arterial pulse and 10-Hz rhythm in SND in different harmonic or complex rational ratios. In each case, the Lissajous diagrams (Figs. 7 and 8, top to bottom) were constructed from consecutive data segments that were 5, 10, and 20 s in length, respectively. The diagrams based on 5-s segments are for the period within an 80-s data block during which the loops were most closely superposed. Lissajous diagrams showing frequency locking in simple harmonic ratios of 1:3, 1:4, and 1:5 appear in Fig. 7, A-C, respectively. Note that the loops in these diagrams remained closely superposed when the period of analysis was increased from 5 to 10 s and then to 20 s. Thus frequency locking in these simple harmonic ratios was reasonably stable. This was less so for complex ratios. The Lissajous diagrams in Fig. 8, A-C, show examples of frequency locking in complex ratios of 2:5, 2:7, and 2:9, respectively. Whereas the loops in the diagrams based on 5-s data segments were closely superposed, the plane partially filled in when the period of analysis was increased to 10 s and then 20 s. Nevertheless, the basic patterns of frequency locking were still discernible in the diagrams constructed from the longer data strips. Importantly, more loops were added to the basic pattern as the period of analysis was increased. This is indicated by increased density (blackening) of the regions of the plane covered by the closely superposed loops.

The ratios of frequency locking of the arterial pulse and the 10-Hz rhythm observed in the current study are boxed in Fig. 1. These ratios were restricted to levels 2–5 on the left side of the Farey tree. With few exceptions (see Fig. 10) the ratio of frequency locking was the same for the inferior cardiac and renal postganglionic nerves. Frequency locking was considered to exist only when the same pattern appeared in each of at least four Lissajous diagrams constructed from consecutive 5-s data segments. The number of cases of frequency locking in different ratios of the arterial pulse and the 10-Hz rhythm in inferior cardiac SND are presented in Fig. 9. Frequency locking was detected in 58 of 74 data blocks obtained from 35 cats. The number of data blocks exceeded the number of cats because the analysis was repeated when heart rate and/or peak frequency in the 10-Hz band of SND changed spontaneously. The bar graph in Fig. 9A presents the ratios in order of increasing rational numbers between 0 and 0.4. The most commonly observed ratio was 1:3 (level 2 of Farey tree). This ratio occurred more often than the combined ratios (1:4 and 2:5) in level 3 (see pie graph in

Fig. 6. Bicoherence functions of original CN discharges in 2 experiments (A and B; same as in Fig. 3). Regions of significant bicoherence (P values $10^{-4}$ to $10^{-9}$ in both A and B) not on the diagonal (line of equal frequency) are for the cardiac-related rhythm, 10-Hz rhythm, and their modulated frequencies (sums and differences). Bicoherence functions were constructed using the same frequency resolution and number of windows as for corresponding AS of CN discharges in Fig. 3.

Fig. 5. Lissajous diagrams relating standardized 1-V sine waves of different frequencies. A: amplitude of 3.0-Hz sine wave is plotted against that of 10.0-Hz sine wave at 10-ms intervals for 5-s period. B: same, but for 3.0- and 10.1-Hz sine waves.
Fig. 7. Three examples (A-C) of frequency locking of the AP and 10-Hz slow waves in CN discharges in simple harmonic ratios. Lissajous diagrams (top to bottom) in each column are based on 5-, 10-, and 20-s digitally filtered data strips. In each case, the normalized amplitudes (scale 0–1.0) of the signals are plotted against each other at 10-ms intervals.

Fig. 8. Three examples (A-C) of frequency locking of the AP and 10-Hz slow waves in CN discharges in complex ratios composed of relatively prime integers (no common divisor). Same format as in Fig. 7.
Fig. 9. Frequency locking of the AP and 10-Hz slow waves in CN activity in different rational ratios (levels 2–5 of Farey tree). A: bar graph. Number of cases is plotted against ratio. Ratios are placed in order of increasing rational numbers between 0 and 1 (scale of fractions is not exact). Number of cases of no detectable frequency locking (‘irrational’ (Irrat) ratios) is also presented. B: pie graph shows cases of frequency locking in different ratios (grouped by Farey level) and no frequency locking (‘irrational’ ratios) as percents of total.

In turn, the combined ratios in level 3 of the tree were more commonly observed than the combined ratios in level 4 (1:5, 2:7, and 3:8), and those in level 4 were more prevalent than the combined ratios in level 5 (2:9, 3:10, and 3:11). Nevertheless, there was not a strict inverse relationship between the level of the Farey tree and the prevalence of particular ratios of frequency locking. For example, there were no recorded instances of low-order 1:2 frequency locking. Moreover, the 2:7 ratio in level 4 of the tree occurred as often as the 2:5 ratio in level 3. In 16 cases, no clear pattern appeared in the Lissajous diagrams. The phase space quickly filled within the 5-s periods of analysis in these cases. Because frequency locking was presumed absent, these ratios are designated as ‘irrational’ in Fig. 9.

Figure 10 shows Lissajous diagrams based on discontinuous 5-s data segments from an experiment in which the characteristics of frequency locking of the 10-Hz rhythmic discharges of the inferior cardiac and renal postganglionic nerves to the arterial pulse were different. In data segment A (Fig. 10), locking of the arterial pulse and the 10-Hz rhythm in a ratio of 2:7 was clearly stronger for the inferior cardiac nerve than for the renal nerve. In data segment B (Fig. 10), frequency locking was absent for both nerves. In data segment C (Fig. 10), strong frequency locking of the arterial pulse and the 10-Hz rhythm in inferior cardiac SND occurred in a ratio of 3:10 rather than 2:7. In contrast, the arterial pulse and 10-Hz rhythm in renal SND were not strongly coupled. Locking in a 3:10 ratio was not observed for the renal nerve in this experiment. In four other experiments, episodes of strong frequency locking of the arterial pulse and the 10-Hz rhythm in one nerve appeared when no locking or only weak locking in the same complex ratio occurred for the other nerve.

Ventricular pacing. In addition to constructing Lissajous diagrams, a strategy that we used to demonstrate frequency locking of the cardiac cycle and the 10-Hz rhythm in SND involved electrical pacing of the ventricle to change heart rate. The idea is that changing heart rate will shift not only the frequency of the cardiac-related rhythm in SND but also that of the 10-Hz rhythm if their respective generators are or become frequency locked. Our experiments were aided by using a table in which frequencies (measured in 0.1-Hz increments) in the cardiac-related and 10-Hz bands were matched for each of the ratios in levels 0–5 of the Farey tree. For example, the table tells us that at a heart rate of 3.0 Hz, the 10-Hz rhythm will have an

Fig. 10. Lissajous diagrams (based on 5-s digitally filtered data strips) from an experiment in which the characteristics of frequency locking of the 10-Hz rhythmic discharges of the CN and renal sympathetic nerve (RN) to the AP were different. Results for 3 discontinuous data segments are shown in A–C. In each diagram, the normalized amplitudes (scale 0–1.0) of the signals are plotted against each other at 10-ms intervals.
actual frequency of 9.0 Hz in the case of 1:3 locking and 10.5 Hz for 2:7 locking. If the frequency of the rhythm in control (no pacing) is much closer to 10.5 than 9.0 Hz, pacing the heart to a rate of 3.0 Hz might induce 2:7 rather than 1:3 frequency locking, despite the greater stability of the lower-order ratio.

The results obtained with ventricular pacing in 11 cats are summarized as follows. There were seven cases in which the ratio of frequency locking was changed from simple harmonic to complex or vice versa. The ratio changed from 1:3 to 2:5 ($n = 1$) or 2:7 ($n = 2$) and from 1:4 to 2:5 ($n = 1$) or 2:7 ($n = 1$) or 2:9 ($n = 2$). There were six cases in which a harmonic ratio was maintained ($1:3, n = 1$) or changed to another harmonic ratio ($1:3$ to 1:4, $n = 2$; 1:3 to 1:5, $n = 1$; 1:4 to 1:5, $n = 2$) during ventricular pacing. There were also five cases in which frequency locking was present at one heart rate and absent at another. The ratios of frequency locking in these experiments were 1:3 ($n = 3$), 1:5 ($n = 1$), and 2:5 ($n = 1$).

Frequency locking of 10-Hz rhythm to raphe stimulation. The question arises whether the power in the 10-Hz band of SND is affected when the rhythm becomes frequency locked to the cardiac cycle and, if so, whether the changes are different for simple harmonic versus complex ratios. A decision on this issue cannot be made on the basis of the experiments with ventricular pacing, because changes in heart rate so induced invariably were accompanied by changes in mean arterial and pulse pressures and thus the strength of pulse-synchronous BNA. For this reason, a different strategy was adopted to test whether frequency locking per se affects the power in the 10-Hz band of SND. Low-intensity electrical activation of sympathoinhibitory sites in the caudal medullary raphe nuclei provides a convenient way to entrain the free-running 10-Hz rhythm in SND of baroreceptor-denervated cats (24). The coupling relationship established is expressed as the ratio between the frequency of raphe stimulation and the frequency of the entrained rhythm. Frequency locking is assumed to be present when raphe stimulation moves the 10-Hz rhythm to a new frequency and a clear pattern appears in Lissajous diagrams. For construction of Lissajous diagrams, the raphe stimulus is represented by a standardized sine wave whose period corresponds to the frequency of stimulation. Raphe stimulus current was kept constant (range, 20–200 µA) for each of the rates of stimulation used in an experimental series.

The results obtained in one experiment are shown in Fig. 12. Autospectral analysis showed that the frequency of the free-running rhythm in SND (no raphe stimulation) of this baroreceptor-denervated cat was 9.0 Hz (Fig. 12A, trace 1). Raphe stimulation at 2.8 Hz moved the frequency of the rhythm to 8.4 Hz (Fig. 12A, trace 2), thus establishing a 1:3 ratio of frequency locking. Frequency locking in this simple harmonic ratio was confirmed by phase plane analysis (Fig. 12B). Note that peak power in the 10-Hz band of SND was increased considerably during 1:3 locking of the rhythm to raphe stimulation. Total power in the 10-Hz band was 110% of control in this case. In contrast, peak
The results obtained with raphe stimulation in 11 baroreceptor-denervated cats are summarized in Fig. 14. Peak power in the 10-Hz band of SND (% of unstimulated control) is plotted against the ratio of frequency locking of the 10-Hz rhythm to the raphe stimulus (ordered in rational numbers between 0 and 1). The total number of observations exceeds the number of cats because more than one ratio of frequency locking was induced in each experiment. In contrast to the results for simple harmonic ratios, peak power in the 10-Hz band of SND was reduced when the ratio of frequency locking was complex. The degree of reduction in peak power of the 10-Hz rhythm, however, was not

Fig. 12. Frequency locking of raphe stimuli (130 µA; 1-ms pulse) and 10-Hz rhythm in CN discharges after baroreceptor denervation. A: trace 1 is AS of CN discharges in absence of raphe stimulation. Traces 2 and 3 are AS of CN discharges during raphe stimulation at 2.8 and 3.4 Hz, respectively. Spectra are based on 32 10-s windows with 75% overlap and are superposed on same power scale. Frequency resolution is 0.1 Hz/bin. B: Lissajous diagram showing frequency locking of raphe stimuli (2.8 Hz) and 10-Hz slow waves in CN discharges in a 1:3 ratio. Raphe stimuli were represented by standardized sine waves whose period corresponds to the frequency of stimulation. Normalized amplitude (scale 0–1.0) of the sine wave is plotted against that of the 10-Hz slow wave (digitally filtered record) at 10-ms intervals for a 5-s period. C: Lissajous diagram showing frequency locking of raphe stimuli (3.4 Hz) and 10-Hz slow waves in CN discharges in a 2:5 ratio.

Fig. 13. Frequency locking of raphe stimuli (St; 100 µA; 1-ms pulse) and 10-Hz slow waves of CN activity in simple harmonic ratios in a baroreceptor-denervated cat. A: AS of CN discharges in the absence (bold traces) and presence (light traces) of raphe stimulation are superposed on the same power scale. Ratios of frequency locking range from 1:1 (top) to 1:5 (bottom). Dotted line in each panel intersects the peak near 10 Hz in the control spectrum. Spectra are based on 32 10-s windows with 75% overlap. Frequency resolution is 0.1 Hz/bin. Frequencies of raphe stimulation were equal to (top) or subharmonics of the frequency of the free-running 10-Hz rhythm. B: Lissajous diagrams showing frequency locking of raphe stimuli and 10-Hz slow waves of CN activity in each of the harmonic ratios between 1:1 and 1:5. Raphe stimuli were represented by standardized sine waves whose period corresponds to the frequency of stimulation. Normalized amplitude (scale 0–1.0) of the sine wave is plotted against that of the 10-Hz slow wave (digitally filtered record) at 10-ms intervals for a 5-s period.

power was decreased (Fig. 12A, trace 3) when the frequency of raphe stimulation was increased to 3.4 Hz. Raphe stimulation at this frequency moved the frequency of the 10-Hz rhythm to 8.5 Hz, thus establishing a 2:5 ratio of frequency locking. Frequency locking in this complex ratio was confirmed by phase plane analysis (Fig. 12C). Total power in the 10-Hz band was decreased to 57% of control in this case.

For frequency locking of the 10-Hz rhythm to raphe stimulation in simple harmonic ratios, the degree of enhancement of peak power in the 10-Hz band was inversely proportional to the denominator of the ratio. In the experiment illustrated in Fig. 13, we used frequencies of raphe stimulation that were subharmonics of or equal to the frequency of the free-running rhythm. In such cases, frequency locking is established (as assessed by phase plane analysis) without moving the frequency of the rhythm. The superposed autospectra of inferior cardiac SND in Fig. 13A clearly demonstrate the inverse relationship between the degree of enhancement of 10-Hz power and the denominator of the simple harmonic ratio of frequency locking. The Lissajous diagrams in Fig. 13B show strong frequency locking for all of the simple harmonic ratios between 1:1 and 1:5.
related to the denominator or the numerator of the complex ratio.

With the aid of the table referred to in Ventricular pacing, we were successful in obtaining the predicted simple harmonic ratio of frequency locking of the 10-Hz rhythm to the raphe stimulus in each of 39 cases attempted. Complex ratios were obtained as predicted in 35 cases. In 14 other cases, however, simple harmonic ratios of frequency locking were induced instead of the predicted complex ratio. In each of these cases, the harmonic ratio was one of the parents from which the predicted complex ratio would have been derived. For example, we induced 1:2 frequency locking in four cases and 1:3 locking in another four cases in place of the predicted 2:5 ratio. The other cases included a 1:1 ratio in place of 2:3 \((n = 1)\), 1:2 in place of 3:7 \((n = 1)\), and 1:3 in place of 3:8 \((n = 3)\) or 2:7 \((n = 1)\).

As expected, Lissajous diagrams showed no signs of frequency locking of the arterial pulse and the 10-Hz rhythm in SND of baroreceptor-denervated cats.

**DISCUSSION**

The Farey tree is a generic mathematical construct that summarizes all possible modes of frequency locking of a nonlinear oscillator to a periodic forcing signal. In the ideal situation, where the system is devoid of noise, the number of modes of frequency locking is infinite (all rational numbers between 0 and 1 qualify). However, the noise in a system limits the number of modes that are practical because the structural stability of frequency locking is low (as defined by the widths of Arnol’d tongues) for high-order ratios. Thus the number of stable modes of frequency locking that can be attained is reduced as noise in the system is increased. This is most telling in biological systems. For example, skilled pianists and drummers can coordinate bimanual hand tapping in only a few low-order rational ratios \((7, 28, 31)\). A somewhat wider range of ratios is possible for frequency locking of the action potentials of isolated cardiac muscle cells to forcing electrical stimuli \((12, 23)\). The current study is the first to provide a detailed analysis of the modes of frequency locking of the 10-Hz rhythm in SND to pulse-synchronous BNA (as reflected by the arterial pulse). We found that these rhythms could be locked under natural conditions (no ventricular pacing) not only in low-order simple harmonic ratios but also in high-order complex ratios extending into level 5 of the Farey tree. Moreover, ventricular pacing changed the frequency of the 10-Hz rhythm as well as heart rate so as to maintain or change the ratio of frequency locking in a predictable way. The dynamics and potential physiological significance of these nonlinear relationships are considered below.

Phase plane analysis revealed frequency locking of the arterial pulse and 10-Hz rhythm in 78% of the cases tested in baroreceptor-innerverated cats. Thus nonlinear coupling of pulse-synchronous BNA and the 10-Hz rhythm in SND was common under the conditions of our experiments. In contrast, the arterial pulse and 10-Hz rhythm were never frequency locked after baroreceptor denervation. The coupling in baroreceptor-innervated cats may have reflected a direct action of elements in the afferent limb of the baroreceptor reflex arc on the brain stem oscillator \((21)\) responsible for the 10-Hz rhythm. In this case, the central networks responsible for this rhythm and the 2- to 6-Hz oscillations in SND would be independently entrained in different ratios to pulse-synchronous BNA. One-to-one coupling of the lower frequency component to pulse-synchronous BNA has been proposed to account for the cardiac-related rhythm in SND \((19, 20)\). It is also possible that entrainment of the 10-Hz rhythm to pulse-synchronous BNA was indirectly mediated via input from the network responsible for the cardiac-related rhythm in SND. As expected, phase plane analysis revealed frequency locking of the 10-Hz rhythm to the cardiac-related rhythm in SND as well as to the arterial pulse. The loops in the Lissajous diagrams relating the cardiac-related and 10-Hz rhythmic discharges of a sympathetic nerve generally were not as closely superposed as those in diagrams relating the arterial pulse to the 10-Hz rhythm. However, it is unclear whether this reflected the greater variability of the amplitude of the cardiac-related slow wave in SND versus that of the arterial pulse (see Fig. 2C) or differences in the strength of frequency locking of these signals to the 10-Hz slow waves. Thus the current study does not distinguish between the two potential pathways (direct and indirect) for frequency locking of the 10-Hz rhythm to pulse-synchronous BNA.

A particularly interesting observation was that the characteristics of frequency locking of the 10-Hz rhythmic discharges of the inferior cardiac and renal nerves to the arterial pulse could be markedly different for short periods (5-s data segments). Phase plane analysis revealed instances of strong locking for one nerve and weak or no locking for the second nerve or frequency
locking of the 10-Hz rhythmic discharges of the two nerve-to-arterial pulse in different rational ratios. These results support our hypothesis (21, 24) that the 10-Hz rhythmic discharges of sympathetic nerves with different targets arise from separate, although usually strongly coupled, brain stem oscillators. Moreover, the data presented here suggest that these oscillators are independently entrained by pulse-synchronous BNA.

Numerical studies of forced oscillators have revealed many of the rules that govern the dynamics of frequency locking in biological and physical systems. The objectives of such studies include the construction of Arnol’d tongues, each of which reflects the stability of frequency locking in a different ratio. For discourses on this subject, the reader is referred to the studies of Allen (1), deGuzman and Kelso (15), Glass and Mackey (23), and Jensen et al. (27). Our experimental data generally adhere to the rules defined by these numerical studies. First, there was more smearing of the patterns in Lissajous diagrams for complex ratios of frequency locking than for simple harmonic ratios. This was apparent in Lissajous diagrams based on 10- or 20-s data segments (see Figs. 7 and 8). This was expected due to the inherently greater stability of frequency locking in low-order simple harmonic ratios than in higher-order complex ratios (28). Second, in combination the low-order ratios of frequency locking (1:3, 1:4, and 2:5) were observed more often than all of the higher-order ratios combined (see Fig. 9). This also is consistent with the inherently greater stability of low-order frequency locking. However, there were no instances of 1:2 frequency locking, which is a ratio in level 1 of the Farey tree. Moreover, 2:7 locking (level 4) was observed as often as 2:5 locking (level 3). We do not believe that these examples break the rules dictated by numerical studies of forced oscillators. Rather, it is likely that our experimental results were strongly influenced by the constraints imposed on the system due to the frequency bands in which heart rate (2–4 Hz) and the 10-Hz rhythm (7–13 Hz) naturally occur (4, 22). For example, 1:2 frequency locking would require a heart rate of 5 beats/s when the rapid rhythm in SND had a frequency of 10 Hz. Heart rates this high are rarely observed in urethane-anesthetized cats, and it is unlikely that a low heart rate could force the free-running rhythm to a frequency <7 Hz. Thus frequency locking in ratios such as 1:3 or 2:7 would be more apt to occur naturally despite the inherently greater stability of 1:2 locking. The constraints imposed by the frequency bands in which heart rate and the 10-Hz rhythm naturally occur probably also explain why all of the coupling ratios observed were on the left side of the Farey tree (see Fig. 1). As expected, however, ratios of frequency locking located on both sides of the tree were observed when raphe stimulation was used to entrain the 10-Hz rhythm in baroreceptor-denervated cats. In these experiments the frequency of raphe stimulation was set at will. Whereas we were invariably successful in establishing the desired simple harmonic ratio of frequency locking of the 10-Hz rhythm to the raphe stimulus, in 14 cases a simple harmonic ratio was obtained instead of the predicted complex ratio. Such instances were consistent with the rule that bifurcations (nonlinear changes in state) of the system are most likely to involve transitions from a high-order to an adjacent low-order ratio of frequency locking (7, 31). For example, in cases when we failed to establish 2:5 locking, either 1:2 or 1:3 locking ratios (the parents of 2:5) were observed instead.

In confirmation of the results obtained by Gebber et al. (22), bicoherence analysis demonstrated quadratic nonlinear coupling (i.e., phase locking) of frequency triples in SND composed of the cardiac-related and 10-Hz rhythms and their modulated frequencies (sum and difference). Phase locking of such frequency triples occurred even when corresponding Lissajous diagrams failed to show frequency locking of the 10-Hz rhythm to the arterial pulse (see Figs. 4B and 6B). Thus statistically significant bicoherence should not necessarily be attributed to the frequency locking of central generators as has been proposed for frequency components in the EEG (5, 17). There are at least two ways to explain phase locking of frequency triples in the absence of frequency locking. First, phase locking of the cardiac-related and 10-Hz rhythms and their modulated frequencies might occur at a level below the generators of the primary frequencies. For example, bulbospinal or spinal sympathetic neurons might act nonlinearly on converging inputs from two independently acting (not coupled) generators to produce modulated frequencies and, thus, phase locking of frequency triples. In this case, the pattern of SND would be quasiperiodic (10, 28), as characterized by an irrational ratio of the cardiac-related and 10-Hz rhythms. Quasiperiodic dynamics occur in those regions of the parameter space located between Arnol’d tongues (1, 15, 23, 27). Second, quasiperiodic patterns might reflect an interaction of the generators of the cardiac-related and 10-Hz rhythms too weak to induce frequency locking but strong enough to generate new modulated frequencies. Nevertheless, the possibility cannot be discounted that the ratio of frequency locking in some cases was too high to be detected in the Lissajous diagram due to the obscuring of an extremely complex loop by noise or variations in the amplitude of the slow waves in the data sample.

To our knowledge, this is the first study to concern itself with relative changes in the amplitude of a forced oscillation when frequency locking occurs in simple harmonic versus complex ratios. Because population recordings were made, the amplitude of the 10-Hz sympathetic nerve slow wave reflects the number of active postganglionic fibers and the degree to which their discharges are synchronized. In these experiments, we substituted raphe stimuli for pulse-synchronous BNA as the forcing signal. This substitution seems appropriate for the following reasons. First, electrical activation of the raphe led to pure sympatho-inhibition and resetting of the 10-Hz rhythm (24) as does pulse-synchronous BNA. Second, the intensity of the raphe stimulus was kept the same at each rate of stimulation in an experimental series. In contrast, changing heart rate by ventricular pacing invariably
affected mean arterial and pulse pressures and, thus, the strength of pulse-synchronous BNA. Third, the regions of the raphe that were stimulated contain neurons with naturally occurring activity correlated to both the cardiac-related and 10-Hz rhythms in SND (2, 3). Fourth, electrical stimulation of the aortic depressor or carotid sinus nerves is inconvenient because activation of sympathoexcitatory chemoreceptor afferent fibers is difficult to avoid in the cat.

Frequency locking of the 10-Hz rhythm to raphe stimulation in simple harmonic ratios increased peak power in this band of SND. At first glance, it seems surprising that electrical activation of sympathoinhibitory sites led to enhanced peak power. We suggest that, as is the case for the displacement of a pendulum by a forcing input received at the same phase in each cycle, the amplitude of the 10-Hz oscillation is increased when the frequency of the rhythm is forced to the rate of raphe stimulation or one of its harmonics. “Same-phase” driving would lead to resonation (i.e., reinforcement) of the 10-Hz oscillation independent of whether the forcing input is excitatory or inhibitory (6). In our experiments, the enhancement of peak power in the 10-Hz band was optimal for the 1:1 ratio of frequency locking, a condition under which the 10-Hz oscillator is forced at the same phase in each cycle. It follows that the degree of enhancement of the 10-Hz oscillation should be inversely proportional to the denominator of the harmonic ratio, a situation also found to exist in our experiments.

In contrast, peak power in the 10-Hz band of SND was decreased when the rhythm was locked to raphe stimulation in complex ratios. It is unlikely that the decrease in power simply reflected the activation of a sympathoinhibitory pathway for two reasons. First, relatively high frequencies of raphe stimulation leading to 1:1 locking enhanced rather than reduced 10-Hz peak power. Second, frequency locking in different complex ratios induced by different frequencies of raphe stimulation led to similar reductions in 10-Hz peak power (see Fig. 14). It is tempting to speculate that the reduction in power was due to the fact that input from the raphe reaches the 10-Hz oscillator at different phases of its cycle when frequency locking occurs in a complex ratio. In the least complicated cases, the number of phases would be equal to the numerator of the ratio (23). Because resetting of the 10-Hz rhythm would have different characteristics for each of the phases, the 10-Hz oscillator might be “destabilized,” thereby leading to a reduction in the average number of active cells in each cycle. However, our data do not fit this simple model because there was no clear relationship between the numerator of the complex ratio and the degree of depression of 10-Hz peak power (see Fig. 14). Thus, the explanation for the reduction in power during frequency locking in complex ratios remains elusive.

Perspectives

We have demonstrated that frequency locking of the 10-Hz rhythm in SND to the cardiac cycle can occur in complex as well as simple harmonic ratios under natural conditions. Coupling is attributed to forcing of the 10-Hz oscillator by pulse-synchronous BNA, and its dynamics generally adhere to the branching structure of a generic mathematical construct called the Farey tree. Intriguingly, in baroreceptor-denervated cats frequency locking in simple harmonic ratios of the 10-Hz rhythm to stimuli applied to raphe sympathoinhibitory sites was accompanied by enhanced power in this band of SND. In contrast, frequency locking in complex ratios composed of relatively prime integers (no common divisor) was accompanied by a reduction in 10-Hz power. Under the assumption that the dynamics of frequency locking of the 10-Hz oscillator to its forcing inputs are universal, these findings raise the possibility that pulse-synchronous BNA also exerts divergent actions on the 10-Hz rhythm in SND depending on the ratio of frequency locking. This possibility and its physiological implications should be the subjects of future investigations. Moreover, an attempt should be made to identify the control parameters and experimental conditions that favor frequency locking in simple harmonic versus complex ratios. The data also revealed that the central circuits responsible for the 10-Hz discharges of different sympathetic nerves can be independently entrained by pulse-synchronous BNA. This finding may also have important physiological implications. For example, instances when frequency locking of the 10-Hz rhythm to the cardiac cycle was strong for one nerve but weak or absent for another nerve may reflect a mechanism based on the resonant properties of forced oscillators by which the central nervous system differentially affects spinal sympathetic outflow to different targets.

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