Nonlinear interactions of slow and rapid rhythms in sympathetic nerve discharge

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Zhong, Sheng, Gerard L. Gebber, Shi-Yi Zhou, and Susan M. Barman. Nonlinear interactions of slow and rapid rhythms in sympathetic nerve discharge. Am. J. Physiol. 275 (Heart Circ. Physiol. 44): H331–H340, 1998.—We used bispectral analysis to characterize the nonlinear interactions of the respiratory-related (RR), cardiac-related (CR), or 10-Hz rhythms in sympathetic nerve discharge (SND) of urethan-anesthetized cats. Bispectral analysis investigates relationships among frequency triples in the same signal (inferior cardiac postganglionic SND) where the third frequency is the sum of the other two due to quadratic nonlinear coupling. Coupling of the RR and CR rhythms leading to the generation of new components (i.e., modulated frequencies) in SND occurred in 84% of the total cases, whereas the incidence was 71% for the RR and 10-Hz rhythms. The occurrence of such nonlinear interactions implies that the RR, CR, and 10-Hz rhythms are carried to common targets by the same postganglionic sympathetic neurons. Furthermore, we suggest that nonlinear interactions leading to the generation of new frequencies in SND may affect end-organ function beyond the level expected in simple cases of linear superposition of the primary rhythms. This suggestion is supported by our observation that strong coupling of the RR and CR rhythms resulted in appreciable power at the modulated frequencies. Bispectral analysis: cardiac-related rhythm; respiratory-related rhythm; 10-Hz rhythm; quadratic nonlinear coupling

METHODS

Experimental subjects and anesthesia. 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), anesthesia in 12 cats was maintained with urethan (1.2–1.8 g/kg iv, initial dose supplemented every 4–6 h with 0.2 g/kg iv). The initial dose range has been reported to maintain a surgical level of anesthesia in cats for 8–10 h (13). The fronto-parietal electroencephalogram (EEG) showed a mixture of 7- to 13-Hz spindles and delta-slow waves, indicative of unconsciousness and blockade of information transfer through the thalamus (25). The EEG was not changed by noxious stimuli (e.g., pinch) applied to the head or body and was not related to SND (4).

General procedures. Blood pressure was measured from a catheter inserted into the abdominal aorta via a femoral artery. Spontaneous respiration during anesthesia was eupneic with end-tidal CO2 (model 2200, Traverse Medical Monitors Capnometer) in the normocapnic range. Subsequently, the animal was paralyzed (galamine triethiodide, 4 mg/kg iv, initial dose), a pneumothoracotomy was performed, and the animal was artificially ventilated with room air enriched with 100% O2. End-tidal CO2 was kept near 4.5% by adjusting the parameters of artificial ventilation. Rectal temperature was kept near 38°C with a heat lamp. Mean arterial pressure was 118 ± 4 mmHg in eight cats with intact baroreceptor and vagus nerves. SND contained a mixture of the RR and CR rhythms in these cats. Bilateral section of the carotid sinus, aortic depressor, and cervical vagus nerves (3) was performed in nine cats, five of which were also used before nerve section.

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Baroreceptor denervation and vagotomy eliminated the CR rhythm in SND. Under these conditions, SND contained a mixture of the RR and 10-Hz rhythms. Mean arterial pressure was $132 \pm 5$ mmHg after baroreceptor denervation and vagotomy.

Neural recordings. As previously described (3, 26), potentials were recorded monophasically with bipolar platinum electrodes from the central ends of the cut left inferior cardiac sympathetic nerve and right phrenic nerve. With the preamplifier band pass set at 0.1–1,000 Hz (3, 9), CR and 10-Hz bursts of SND appeared as slow waves (i.e., envelopes of spikes). The same band pass was used to record phrenic nerve activity (PNA). The frontal-parietal EEG was recorded with a gold-plated disk electrode placed on the skull and the indifferent electrode on crushed muscle; the preamplifier band pass was 1–1,000 Hz.

Data analysis. The original records of SND and PNA were sampled at 100 Hz after low-pass filtering at 50 Hz in preparation for fast Fourier transform (FFT). The analog filter (model AP 260–5, Avens) had an attenuation slope of 24 dB/octave. The same sampling rate was used for the arterial pulse (AP). Autospectra of SND, PNA, and the AP and coherence functions measuring the linear correlation strength (scale 0–1.0) of pairs of these signals were computed by using a modified version (21) of the program of Cohen et al. (10). FFT was performed on 32 10-s windows with 50% overlap (165-s data blocks). The resolution of measurement was 0.1 Hz/bin. A coherence value of $\geq 0.1$ reflects a significant linear correlation between two signals when 32 windows are averaged (7).

The bicoherence function (normalized bispectrum) of SND was computed using a modified version (14) of the program of Dunemuth and Gasser (11). Full details as well as the formulas for calculation of the bicoherence function are given in Refs. 14 and 18. The data windows analyzed were the same as those used to calculate the autospectrum. The bicoherence values describing the strength of quadratic nonlinear coupling of frequency triples composed of primary (e.g., RR and CR rhythms) and modulated frequencies are compared with idealized values for predefined probability levels under a Gaussian hypothesis (See Subroutine Bicoh on p. 237–238 in Ref. 14). The comparison allows one to decide whether the frequency components in SND are statistically independent (i.e., linearly superposed) or nonlinearly interactive. A P value of $10^{-4}$ is used as the threshold for significant coupling of a frequency triple. This threshold reflects the 95% confidence limits of the biphase, which serves as a second measure of the strength of coupling (12). The absolute value of the biphase is a measure of the shifting of the origin of the higher frequency (e.g., CR rhythm) with respect to the lower frequency (e.g., RR rhythm). Although absolute values are not considered in this study, the 95% confidence limits of the biphase were less than or equal to $\pm 20^\circ$ for a P value $= 10^{-4}$. By comparison, the 95% confidence limits were much wider ($=110^\circ$) for $P > 10^{-4}$.

Nonlinear frequency locking of two rhythms in a rational ratio of integers is a special case leading to the generation of modulated frequencies and thus quadratic coupling of frequency triples (8, 14, 15). In the current study, phase-plane analysis was used to test for frequency locking of the RR and CR rhythms in SND. This entailed the construction of Lissajous diagrams in which the voltages of pairs of signals are repeatedly sampled and plotted against each other on the x- and y-coordinates of a plane. Frequency locking exists when successive loops in the Lissajous diagram are similar in form and are closely superposed. In contrast, no clear pattern appears when frequency locking is absent. Rather, the plane quickly fills in due to the sliding relationship between the signals. The method described below is based on the protocol used by us in an earlier study (15) to identify ratios of frequency locking of the CR and 10-Hz rhythms in SND. 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 on the shape of the loops relating pairs of signals. For SND, two copies of the original signal are independently digitally filtered using band passes encompassing the RR and CR rhythms. The width and center frequency of the band pass for the RR rhythm matched those of the peak in the original autospectrum of SND at the frequency of respiration. For the CR rhythm in SND, the width and center frequency of the band pass matched those of the peak in the autospectrum of SND at the frequency of the heartbeat. The filtered signals are smooth and more sinusoidal-like than the originals with power reduced by no more than 15% in the designated band pass. The roll-off slope of the filter was such that power outside of the band pass was reduced by 39% Hz. PNA and the AP are also digitally filtered using the same band passes as for the RR and CR sympathetic rhythms, respectively. Lissajous diagrams are constructed by plotting the voltages of pairs of signals against each other at successive 10-ms intervals for a period ($\sim 21.5$ s in Fig. 5) containing eight or more respiratory cycles. The program used (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 plane. Additional details are given in the description of Fig. 5 in RESULTS.

The Fisher's "Exact Test" (24) was used to compare the relative incidences of significant nonlinear coupling of the RR and CR versus RR and 10-Hz rhythms. The Mann-Whitney nonparametric test (24) was used to determine whether the sum of peak powers in the RR and CR bands or RR and 10-Hz bands of SND (expressed as a percentage of total power) was related to the strength of nonlinear coupling of these rhythms. Values in the text are means $\pm$ SE.

RESULTS

RR and CR rhythm interactions. Figure 1A (top to bottom) shows oscilloscopic traces of the AP, PNA, and SND in a baroreceptor-innervated cat with intact vagus nerves. Note that SND contained a mixture of slow and rapid rhythms corresponding to the frequencies of phrenic nerve bursting (inspiration) and the heartbeat. The RR rhythm in SND was characterized by slow cyclic changes in baseline and the amplitude of the CR slow waves. CR slow-wave amplitude was maximal during the inspiratory phase of PNA. Although not shown, ganglionic blockade with hexamethonium chloride (10 mg/kg iv) eliminated inferior cardiac nerve activity (i.e., SND trace flattened to a line).

Figure 2, left (top to bottom), shows the autospectra of SND, PNA, and AP from the same experiment as in Fig. 1A. The autospectrum of SND contained two large peaks: the first at the frequency (0.4 Hz) of the central respiratory cycle (primary peak in PNA autospectrum) and the second at the frequency (3.5 Hz) of the heartbeat (primary peak in AP autospectrum). Coherence functions measuring the strength of linear correlation between pairs of these signals are also shown in Fig. 2. SND and PNA were strongly correlated (peak coher-
ence value, 0.90) at the frequency of the central respiratory cycle (Fig. 2, top right). SND and the AP were significantly coherent (Fig. 2, middle right) at the frequencies of the respiratory cycle, heartbeat, and second harmonic of the heartbeat. Coherence of SND to the AP at the frequency of the respiratory cycle indicates that the AP contained some power at this frequency even though it was undetectable on the scale used to construct its autospectrum. Because the coherence function is the normalized cross-spectrum, its value at any frequency is mathematically independent of the absolute powers in the paired signals (6). PNA and the AP cohered significantly (Fig. 2, bottom right) at the frequency of the respiratory cycle but not at the frequency of the heartbeat.

Figure 3A, bottom, shows the bicoherence function of SND from the same experiment as in Figs. 1A and 2. It should be read as follows. Frequencies $f_1$ and $f_2$ are plotted symmetrically as x- and y-coordinates. The shaded regions denote statistically significant quadratic nonlinear coupling of frequency triples composed of $f_1$, $f_2$, and their sum. These regions represent peaks that rose above the Gaussian noise (white background). The strength (amplitude of peak) of coupling of a frequency triple is coded by a $P$ value. A $P$ value of $10^{-4}$ was used as the threshold for significant coupling (see METHODS). The $P$ values cited in the text are provided by digital readout. In picture form, $P$ values are represented by the pattern of black dots on the white background (see scale in Fig. 3A). The significant bicoherence levels ($P < 10^{-9}$) arising from Gaussian noise plotted symmetrically near x- and y-coordinates 0.4 and 3.5 Hz reflect coupling of the RR and CR rhythms, respectively, and their sum (a modulated frequency). The significant bicoherence levels ($P = 10^{-8}$ to $< 10^{-9}$) near x- and y-coordinates 0.4 Hz and 3.1 Hz reflect coupling of the RR rhythm, the difference between the CR and RR rhythms (also a modulated fre-
quency), and their sum, the CR rhythm. A third region
of significant bicoherence \( (P \leq 10^{-9}) \) plotted
symmetrically near x- and y-coordinates 0.4 Hz and 2.7
Hz reflects coupling of the RR rhythm, the difference
between the CR rhythm and twice the frequency of the
RR rhythm (yet another modulated frequency), and
their sum. Note that peaks of appreciable amplitude
are present in the autospectrum of SND at the modu-
lated frequencies of 2.7, 3.1, and 3.9 Hz (Fig. 3
A, top).

Regions of significant bicoherence on or near the line
of equal frequency also appear in Fig. 3A, bottom. The
shaded region at x- and y-coordinates 3.5 and 3.5 Hz
reflects nonlinear coupling \( (P \leq 10^{-9}) \) of the CR rhythm
to its second harmonic, thereby indicating that the
cardiac-related slow wave in SND was not a pure
sinusoid (5). The significant bicoherences plotted sym-
metrically near x- and y-coordinates 3.5 and 3.1 Hz and
3.5 and 3.9 Hz reflect coupling of the CR rhythm to
modulated frequencies. Finally, significant bicoherence
near x- and y-coordinates 0.4 and 0.2 Hz reflects
coupling of the RR rhythm to a subharmonic of this
rhythm.

Figure 3B, bottom, shows the bicoherence function of
SND in an experiment in which the frequencies of the
RR and CR rhythms were 0.2 and 3.1 Hz, respectively
(see autospectrum, Fig. 3B, top). In this case, the two
rhythms were not nonlinearly coupled \( (P > 10^{-4}) \). The
only region of significant bicoherence was at x- and
y-coordinates 0.2 and 0.2 Hz \( (P = 10^{-6} \text{ to } 10^{-9}) \). This
region is for the RR rhythm and its second harmonic.
The bar graph in Fig. 4A shows the distribution of P
values reflecting the strength of quadratic nonlinear
coupling of the RR and CR rhythms and their modu-
lated frequencies. The data were obtained from eight
baroreceptor-innervated cats with intact vagus nerves.
The number of cases \( (n = 25) \) exceeded the number of
cats, because the analysis was repeated when the
frequency of the CR rhythm changed spontaneously.
Bicoherence values reaching the highest levels of statis-
tical significance \( (P \leq 10^{-9}) \) were observed in 60% of the
cases analyzed. NS in the bar graph signifies cases of no
nonlinear coupling \( (P > 10^{-4}) \).
In the 25 cases analyzed, the frequencies of the RR and CR rhythms were 0.47 ± 0.04 and 2.98 ± 0.11 Hz, respectively. The RR peak in the autospectrum of SND was 2.1 ± 0.3 times as large as the CR peak. The sum of the peak powers in the single bins at the frequencies of the central respiratory cycle and heartbeat was 12.5 ± 0.8% of the total power (0–50 Hz band) in SND for cases in which bicoherence was nil or significant at \( P = 10^{-4} \). The corresponding value was 20.9 ± 1.0% for cases in which bicoherence was significant at \( P = 10^{-6} \). The percentages for the “nil or weakly coupled” and “more strongly coupled” groups were significantly different (\( P = 0.0032 \)) as determined by using the Mann-Whitney nonparametric test.

Figure 5 shows the Lissajous diagrams from an experiment (same as in Fig. 3A) in which the bicoherence values relating the RR and CR rhythms in SND reached statistical significance at the highest levels (\( P = 10^{-9} \)). Each diagram was constructed from the pair of digitally filtered signals (see METHODS) shown on the left of Fig. 5. All four signals (RR SND, CR SND, PNA, and AP) are from the same data block containing approximately eight respiratory cycles. The loops in the Lissajous diagram (Fig. 5A, right) relating RR SND (plotted on x-axis) to PNA (y-axis) are reasonably closely superposed and are restricted to only a portion of the total phase plane. This reflects 1:1 frequency locking of the slow rhythmic components of these signals, which was expected on the basis of their strong linear coherence at the frequency of the respiratory cycle (Fig. 2, top right). As expected, the loops in the Lissajous diagram (Fig. 5B, right) reflecting the 1:1 relationship of CR SND (x-axis) to the AP (y-axis) also did not fill the total phase plane. There is more smearing in this diagram due, in large part, to waxing and waning of the amplitude of the CR sympathetic nerve slow wave during the phases of the respiratory cycle. Careful inspection reveals that this diagram is composed of two families of loops, one for small amplitude CR slow waves and the second for larger amplitude CR slow waves. In contrast to the Lissajous diagrams in Fig. 5, A and B, the loops in the diagrams relating RR SND to CR SND (Fig. 5C, right) and PNA to AP (Fig. 5D, right) filled much more or all of the phase plane. This implies the absence of frequency locking of these pairs of signals. This support is provided by viewing separately some of the individual loops that made up the composite diagrams.

Three individual loops relating RR SND (x-axis) to CR SND (y-axis) are shown in Fig. 6A. The loops correspond from top to bottom to RR SND cycles 1, 3, and 6 in Fig. 5C, left. Each of the loops evolves from left to right (solid line) on the x-axis and then reverses direction from right to left (dashed line) returning near to the starting point. Note that the shapes of the three loops are quite different, with peaks and valleys shifting in position from diagram to diagram. These differences imply that the relationship between the RR and CR slow waves in SND was continually sliding (not frequency locked). The individual loops themselves are complex in shape for several reasons. First, on the average, there were 8.75 CR slow waves per RR slow wave. Second, the distances between the peaks in each diagram were not uniform. This reflects changes in the slope of RR SND more so than changes in the period of the CR slow wave, which were minimal. In cycles 1 and 6, the tops of the RR slow waves were broad. As a consequence, some of the excursions on the y-axis reflecting CR slow waves were “bunched” together, because they occurred at a time when voltage on the x-axis changed minimally. Third, each loop is further complicated by the progressive increase in amplitude of the peaks as the plot is followed from left to right. Amplitude of the peaks further increased and then decreased on the return trip from right to left. These changes reflect the waxing and waning of CR slow waves, with maximum amplitude occurring during inspiration. This complication is minimized by plotting the relatively constant amplitude AP in place of CR SND on the y-axis. In such diagrams (Figs. 5D and 6B), we also plotted the more stable recording of PNA in place of RR SND on the y-axis. Because the peak of the PNA slow wave was consistently sharp, the “bunching” problem referred to above was eliminated. Despite the relative constancy of the AP and PNA waveforms, the individual loops in Fig. 6B were quite different with regard to positions of peaks and valleys. As a consequence, the phase plane of the Lissajous diagram was completely filled within eight respiratory cycles (Fig. 5D, right). Thus the relationship between PNA and the AP also was sliding rather than fixed.
Phase-plane analysis was performed in five experiments under conditions when bicoherence values relating RR and CR rhythms in SND reached a significance level of $P \leq 10^{-9}$. Four to six separate data segments (each containing 8 respiratory cycles) were analyzed in each of these experiments. In no case did the Lissajous diagrams relating RR SND and CR SND or PNA and AP contain closely superposed loops. Rather, the phase plane was completely or near completely filled within eight respiratory cycles leading to Lissajous diagrams like those shown in the right side of Fig. 5, C and D.

RR and 10-Hz rhythm interactions. Figure 1B shows oscilloscopic traces from a baroreceptor-denervated and vagotomized cat in which SND contained a mixture of the RR and 10-Hz rhythms. The trace of SND was flat after ganglionic blockade with hexamethonium chloride, 10 mg/kg iv (not shown). Thus the slow oscillation in SND with the period of the rhythm in PNA cannot be attributed to movement of the chest. This was unlikely anyway because PNA and the cycle of artificial ventilation are uncoupled by bilateral vagotomy.

Figure 7 shows the autospectra of SND, PNA, and the AP and corresponding coherence functions from another baroreceptor-denervated and vagotomized cat. The autospectrum of SND (Fig. 7, top left) contained two sharp peaks: the first was at the frequency (near 0.5 Hz) of the rhythm in PNA (Fig. 7, middle left) and the second near 10 Hz. The slow rhythms in SND and PNA were significantly correlated (peak coherence value was 0.95; Fig. 7, top right). As expected after baroreceptor denervation (3, 14), SND and the AP were not coherent at the frequency of the heartbeat (Fig. 7, middle right). In this experiment, there was no sign of an RR rhythm in blood pressure, because the AP did not
cohere to PNA at the frequency of the central respiratory cycle (Fig. 7, bottom right).

Figure 8A, bottom, shows the bicoherence function of SND from the same experiment as in Fig. 7. There are two adjacent regions of significant bicoherence ($P = 10^{-5}$ to $10^{-9}$) involving the RR and 10-Hz rhythms and their modulated frequencies. The shaded region near x- and y-coordinates 0.5 and 9.0 Hz plotted symmetrically reflects coupling of the RR and 10-Hz rhythms and their sum. The shaded region near 0.5 and 8.5 Hz reflects coupling of the RR rhythm, the difference between the 10-Hz and RR rhythms, and their sum, the 10-Hz rhythm. Figure 8A, bottom, also shows regions of significant bicoherence on or near the diagonal line of the types previously described. The autospectrum of SND in this experiment (Fig. 8A, top) is typical in that it does not contain distinct peaks of appreciable power at the modulated frequencies. Such peaks were present in only two cases of significant coupling of the RR and 10-Hz rhythms with $P \leq 10^{-5}$.

Figure 8B, bottom, shows the bicoherence function of SND from an experiment in which the RR and 10-Hz rhythms were not quadratically coupled. Note the absence of significant bicoherence at x- and y-coordinates corresponding to the frequencies of the two rhythms that were near 0.6 and 10.4 Hz (see autospectrum in Fig. 8B, top).

The bar graph in Fig. 4B shows the distribution of $P$ values reflecting the strength of nonlinear coupling of the RR and 10-Hz rhythms and their modulated frequencies. Thirty-two data blocks from nine baroreceptor-denervated and vagotomized cats were analyzed. The analysis was repeated in individual experiments when the frequencies of the RR and/or 10-Hz rhythms changed spontaneously. Coupling of these components of SND was observed in 71% of the total cases. However, coupling at $P \leq 10^{-9}$ was seen in only 13% of the cases analyzed compared with 60% of the cases for the RR and CR rhythms (Fig. 4A). This difference was statistically significant ($P = 0.0156$) as determined by using the Fisher's exact test.

In the 32 cases analyzed, the frequencies of the RR and 10-Hz rhythms were $0.40 \pm 0.03$ and $9.02 \pm 0.25$ Hz, respectively. The RR peak in the autospectrum of SND was $5.3 \pm 1.0$ times as large as the 10-Hz peak. The sum of the peak powers in the single bins at the frequencies of the central respiratory cycle and the 10-Hz rhythm was $8.0 \pm 0.7\%$ of the total power in SND for cases in which bicoherence was nil or significant at $P = 10^{-3}$ or $10^{-5}$. The corresponding value was $17.9 \pm 1.8\%$ for cases in which bicoherence was significant at $P \leq 10^{-6}$. The percentages for the "nil or weakly coupled" and "more strongly coupled" groups were significantly different ($P = 0.0002$) as determined by using the Mann-Whitney nonparametric test.

DISCUSSION

Statistically significant bicoherence provides formal proof of nonlinear interactions leading to the generation of new components (i.e., modulated frequencies) in the signal under consideration (11, 12, 14, 18). Importantly, linear superposition of independent rhythms leading to amplitude modulation (i.e., beats) but no new frequencies is reflected by bicoherence values that are not significantly different from zero (see p. 177 in Ref. 23). With these points in mind, our results have revealed a high incidence of nonlinear coupling of frequency triples in SND composed of the RR and CR or 10-Hz rhythms and their modulated frequencies. This finding raises two issues. The first issue concerns the physiological relevance of the nonlinear interactions, and the second concerns the mechanisms responsible for the nonlinearities.

With regard to the physiological relevance of our findings, quadratic nonlinear coupling of frequency triples implies that they are carried, at least in part, to common targets by the same postganglionic sympathetic neurons. This conclusion is based on the assumption that convergence of the primary rhythms is required at some point central to the recording site in order for new modulated frequencies to be generated. Whereas the discharges of some single postganglionic sympathetic neurons have been reported to contain...
both RR and CR components (16), the current study is the first to provide evidence that the RR and 10-Hz rhythms are also carried by the same sympathetic neurons. Moreover, because the CR and 10-Hz rhythms and their modulated frequencies are often nonlinearly coupled (14, 15), it is likely that the discharges of at least some postganglionic sympathetic neurons carry all three rhythms (RR, CR, and 10-Hz) to the same targets.

In those experiments in which the RR and CR rhythms were strongly nonlinearly coupled \( (P < 10^{-9}) \), the sum of the amplitudes of the peaks in the autospectrum of SND at the modulated frequencies was \( \sim 25\% \) of the sum of the amplitudes of the peaks at the two primary frequencies. Thus the power generated at new frequencies by the nonlinear interaction of the RR and CR rhythms could be appreciable. Under the assumption that the overall level of SND is increased by the generation of new frequencies, it seems reasonable to suggest that nonlinear interactions may lead to end-organ responses that are larger than those associated with linear superposition of the primary rhythms. This possibility should be examined in future studies.

Quadratic nonlinear coupling of the RR and CR rhythms in SND was generally stronger than for the RR and 10-Hz rhythms. This was indicated by three observations. First, there was a significantly higher incidence of bicoherence of the RR and CR rhythms at \( P \leq 10^{-9} \) (60% of total cases) than for the RR and 10-Hz rhythms (13% of total cases). Second, there was a higher incidence of linear superposition (nil bicoherence) of the RR and 10-Hz rhythms (28% of total cases) than for the RR and CR rhythms (16% of total cases). Third, distinct peaks of appreciable amplitude in the autospectrum of SND at modulated frequencies were rare in cases of significant bicoherence of the RR and 10-Hz rhythms. Because the power at the new frequencies was minimal, the physiological relevance of the nonlinear interaction of the RR and 10-Hz rhythms is problematical under the conditions of our experiments.

There was significantly greater combined peak power (expressed as a percentage of total power) in the RR and CR or RR and 10-Hz bands of SND when coupling of these rhythms was strong \( (P \leq 10^{-6}) \) rather than nil \( (P > 10^{-4}) \) or weak \( (P = 10^{-4} \) to \( 10^{-5}) \). This observation can be interpreted in at least two ways. First, quadratic nonlinear coupling may strengthen as the interacting rhythms become more prominent. Second, the nonlinear interaction of the rhythms may lead not only to the generation of new frequencies but also to the enhancement of power at the primary frequencies. Currently, we cannot distinguish between these possibilities.

There are at least three ways to explain nonlinear interactions leading to the generation of modulated frequencies in SND. First, the central generators of the RR and CR rhythms or RR and 10-Hz rhythms might become frequency locked. Frequency locking of nonlinear oscillators occurs in rational ratios of integers (2, 8, 19, 20), and the resulting pattern of SND is characterized as periodic (8, 20). Second, modulated frequencies might be generated at a level below the generators of the primary frequencies. For example, bulbospinal or spinal sympathetic neurons might act nonlinearly on converging inputs from independently acting generators to produce modulated frequencies and thus
quadratic coupling of frequency triples. In this case, the ratio of the primary frequencies is irrational and the pattern of SND is characterized as quasiperiodic (8, 20). Third, quasiperiodic patterns of SND might reflect a nonlinear interaction of the generators of the primary rhythms too weak to induce frequency locking but strong enough to generate new frequencies.

In a previous study from our laboratory (15) on the interactions of the CR and 10-Hz rhythms, it became clear that bicoherence analysis alone does not distinguish between periodic and quasiperiodic patterns of SND. The primary objective of this study was to identify ratios of frequency locking of the central generators of these rhythms. For this purpose, Lissajous diagrams relating the CR and 10-Hz rhythms were constructed. Numerous cases of frequency locking in rational ratios ranging from 1:3 to 3:10 were observed (see Figs. 7 and 8 in Ref. 15). As expected, bicoherence analysis demonstrated quadratic coupling of the CR and 10-Hz rhythms and their modulated frequencies in each of these cases. However, statistically significant quadratic coupling was also present in many cases when the Lissajous diagram showed no sign of frequency locking of the primary rhythms (i.e., phase plane filled in; see Figs. 4B and 6B in Ref. 15). This situation applied as well to the RR and CR rhythms in the current study. That is, the phase plane of the Lissajous diagrams relating RR and CR slow waves in SND or PNA and the AP quickly filled in even when the highest levels (P $\leq 10^{-9}$) of significant bicoherence of the RR and CR rhythms were reached. In retrospect, this is not so surprising because numerical studies of nonlinear oscillators have demonstrated that the stability of frequency locking in rational ratios of 1:4 or higher order is inherently low (2, 19, 20). In this regard, the average frequency of the CR rhythm was 6.3 times higher than that of the RR rhythm. It is problematical whether frequency locking in ratios of 1:6 or higher order would be stable enough to account for bicoherence values reaching statistical significance at P $\leq 10^{-9}$. It is more likely that other mechanisms such as the convergence onto common follower neurons of inputs from independently acting oscillators accounted for the nonlinear interactions leading to the generation of new frequencies. In this case, the ratio of the primary frequencies would be irrational leading to a quasiperiodic signal of the type described by Berge et al. (see Fig. III.7 in Ref. 8). Nevertheless, it cannot be denied that in some cases frequency locking may have occurred in too high a ratio to be easily detected in Lissajous diagrams. As shown in Fig. 6, the loops in the Lissajous diagrams relating RR and CR rhythms in SND or PNA
to the AP were extremely complex in shape. Filling in of the x- and y-plane of the Lissajous diagram over the course of the eight respiratory cycles on occasion might have reflected variations in the phase angle of frequency locking rather than the absence of frequency locking in a high-order ratio. Even more complex loops would have been expected in Lissajous diagrams relating the RR and 10-Hz rhythms in SND, because the average frequency of the latter rhythm was 22.5 times higher than that of the former. For this reason, such analysis was not attempted.

In summary, our experiments have revealed a high incidence of quadratic nonlinear coupling of the slow (RR) and rapid (CR, 10-Hz) rhythms in SND, leading in some cases to the generation of appreciable power at new frequencies. The possibility is raised that such nonlinear interactions can lead to end-organ responses that are larger than those associated with linear superposition of the primary rhythms. At the very least, the observed nonlinear interactions imply that the RR, CR, and 10-Hz rhythms are carried to the same targets by the same postganglionic sympathetic neurons. The mechanisms accounting for the nonlinear interactions remain in question. Although phase-plane analysis failed to reveal clear-cut examples of frequency locking of the primary rhythms, we are reluctant to rule out a role for this mechanism in every case. In fact, Höyer et al. (17) and Schäfer et al. (22) have observed low-order ratios (e.g., 1:3, 2:5) of frequency locking of respiration and the heartbeat in humans.

The authors thank Mickie Vanderlip for typing the manuscript. This study was supported by National Heart, Lung, and Blood Institute Grant HL-13187. Address for reprint requests: G. L. Gebber, Dept. of Pharmacology and Toxicology, B426 Life Science Bldg., Michigan State University, East Lansing, MI 48824-1317.

Received 11 February 1998; accepted in final form 7 April 1998.

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