Respiratory modulation of human autonomic rhythms

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BREATHING IS A READILY OBSERVED human rhythm that exerts profound influences on autonomic neural outflow. Not surprisingly, humans figure importantly as experimental subjects for research into respiratory-autonomic interactions. Alone among research subjects, they can cooperate and they can breathe more slowly or rapidly, more shallowly or deeply, or they can hold their breath and not breathe at all. Numerous authors (17, 18, 45) have used controlled respiration as a means to perturb and study important human autonomic mechanisms.

Notwithstanding the advantages humans bring as experimental subjects, they also bring a major disadvantage: they are, perforce, closed-loop preparations in whom fluctuations of recorded signals do not necessarily betray their mechanisms. For example, because arterial pressures and R-R intervals fluctuate in parallel at low and respiratory frequencies, these fluctuations have been considered to be causally related and to reflect baroreflex physiology; arterial pressure changes provoke R-R interval changes (11). However, human time series contain no intrinsic information regarding whether the fluctuations result from actions of independent oscillators or reflexes, and cross-spectral analyses do not even indicate which fluctuation precedes the other. In this study, we asked healthy young subjects to breathe in different ways (or to not breathe at all) as a means to study and better understand how respiration influences human autonomic and hemodynamic rhythms.

METHODS

Subjects. Eight men and one woman, ages 24—35 yr, participated in this study. All subjects were healthy and nonsmokers. Subjects refrained from imbibing alcohol or caffeine-containing drinks, and they did not perform strenuous exercise 24 h before each study. This study was approved by the human research committees of the Medical College of Virginia at Virginia Commonwealth University and the Hunter Holmes McGuire Department of Veterans Affairs Medical Center. Subjects gave their informed, written consent before they participated.

Measurements. We recorded data on digital tape (TEAC RD-145T; Montebello, CA), and subsequently redigitized them at 500 Hz with commercial hardware and software (WINDAQ, Dataq Instruments; Akron, OH). We continuously measured electrocardiographic R-R intervals, respiration (abdominal bellows connected to an uncalibrated strain-gauge pressure transducer), and finger photoplethysmographic arterial pressure (model 2300, Finapres, Ohmeda; Englewood, CO). (In some subjects, we also recorded tidal

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volume with a Fleisch pneumotachograph.) Subjects’ left arms rested in a sling with their hands placed at the level of the right atrium. An infrared analyzer (Gambro; Engstrom, Sweden) measured CO₂ concentrations in samples withdrawn continuously from a face mask. We did not attempt to control subjects’ tidal volumes.

We recorded muscle sympathetic nerve activity directly (model 662C-1, Nerve Traffic Analyzer, University of Iowa Bioengineering; Iowa City, IA), as described previously (71). Briefly, we made our recordings with a tungsten microelectrode (FHC; Bowdoinham, MA) with a ~2- to 5-μm uninsulated tip, inserted into the right peroneal nerve, posterior to the fibular head. The microelectrode was used first to deliver electrical stimuli to provoke muscle twitches and then to record nerve signals. An uninsulated reference electrode was inserted 2–3 cm from the recording electrode. Both electrodes were connected to a differential preamplifier and to an amplifier (total gain of 70,000) where the nerve signal was band-pass filtered (700–2,000 Hz) and then integrated (time constant 0.1 s) to obtain mean voltage neurograms.

Recording electrodes situated in mixed peripheral nerves register all traffic, afferent as well as efferent; however, only muscle and skin sympathetic nerve traffic occurs as pulsatile bursts. These bursts of nerve activity are efferent, not afferent, because they are abolished by proximal, but not distal, anesthetic injections; they arrive at the recording site after ganglionic transmission because they are reversibly abolished by ganglionic blocking agents; and they are succeeded by changes of sympathetic effector function, including increases of arterial pressure or skin resistance (12, 29). The challenge is to determine whether such sympathetic bursts travel to muscle or skin vascular beds. We used the following criteria to differentiate muscle from skin sympathetic nerve activity: 1) bursts were pulse synchronous with the electrocardiogram; 2) Valsalva maneuvers increased burst frequency and size; 3) muscle stretch increased afferent nerve traffic; 4) light skin stroking did not increase afferent nerve activity; and 5) loud noises did not affect activity. Nerve signals were monitored throughout each experiment on a computer terminal.

**Experiment protocol.** Subjects remained supine throughout the research protocol and breathed in the following fixed sequence: 1) spontaneous, uncontrolled breathing for 5 min; 2) controlled fixed-frequency breathing at 0.25 Hz for 5 min; 3) hyperventilation with 100% oxygen for 2 min; followed by 4) inspiratory breathing holding for as long as possible; and 5) loud noises did not affect activity. Nerve signals were monitored throughout each experiment on a computer terminal.

**Data analysis.** We analyzed our results with custom software, WinCPRS, developed by one of us (T. A. Kuusela, Turku, Finland).

**Sympathetic nerve activity.** The WinCPRS program automatically detects sympathetic bursts with signal-to-noise ratios >3:1 and the time from the preceding (one removed) R wave to each sympathetic burst peak occurring at 1.3 ± 0.50 s (21). Two observers manually over-read results of automated analyses, removed erroneously detected bursts, and added bursts that were missed by the computer program. The integrals of all sympathetic burst areas and amplitudes occurring during the initial 5-min period of uncontrolled breathing were divided by the number of bursts to derive an average control burst area and amplitude. The area and amplitude of each sympathetic burst detected subsequently were divided by these numbers to derive normalized burst areas and amplitudes. (For example, if the area of a burst were twice as large as the area of the average control burst, its normalized area would be 2.0.) We expressed sympathetic nerve activity as bursts per minute and total activity (bursts/min multiplied by average normalized burst areas). For spectral analysis of the discontinuous sympathetic nerve signal, we averaged sympathetic nerve activity over each second.

We measured average values and transients during each data segment. For each breathing protocol, we averaged responses over 1) the total duration; 2) the first and last 2 min; 3) the first and last minute; and 4) every 15 s. We also averaged 30-s epochs every 5 s at the beginning and end of apnea and at the beginning of hyperventilation. (We could not average responses of our subjects throughout apnea, because the durations of apnea varied widely. However, because the shortest duration of apnea was 3 min, we were able to average the first 3 min and back-average the last 3 min of apnea for all subjects.)

**Power spectra.** We used fast Fourier transformation to calculate spectral power of the R-R interval, systolic and diastolic pressures, and muscle sympathetic nerve activity time series, as described previously (8). Briefly, the waveforms were linearly interpolated and resampled at 5 Hz. They were then passed through a low-pass impulse response filter with a cut-off frequency of 0.50 Hz. Thirty-second (300 samples) data sets, sliding every 10 s, were detrended, Hann-filtered, and fast Fourier transformed to their respective frequency representations. We used a periodogram method to estimate power distribution (49). The areas under the low (0.05–0.15 Hz) and respiratory frequency (0.15–0.30 Hz) peaks were integrated and averaged for all subjects. Muscle sympathetic nerve spectral power was characterized both in terms of frequency and normalized burst areas and amplitudes.

**Baroreflex.** We estimated vagal baroreflex gain at low frequencies from the squared coherence between pairs of measurements by dividing the squared cross-spectral densities of systolic pressures and R-R intervals by the product of the individual power spectral densities and the phase angle as the arc tangent of the quotient of the quadrature and coincident spectral density functions. The transfer function was calculated as the quotient of the cross-spectral density and the power spectral density of the systolic pressure. The modulus of the transfer function was used to estimate baroreflex gain (58).

**Partial coherence.** Partial coherence analysis is a technique that mathematically removes the influence of one signal (in our case, respiration) from two other signals (systolic pressure and R-R intervals), and then determines coherence between the residual components of the other two signals. The partial coherence function of the input signals have the index 1 and 2, and the output signal has the index y) was calculated as

\[
\gamma^2_{2y-1}(f) = \frac{(G_{2y-1}(f))}{G_{22-1}(f)G_{y-1}(f)}
\]

where

\[
G_{2y-1}(f) = G_{2y}(f) - \frac{G_{21}(f)}{G_{11}(f)} G_{1y}(f)
\]

\[
G_{22-1}(f) = G_{22}(f) - \frac{G_{21}(f)}{G_{11}(f)} G_{12}(f)
\]
We discuss this assumption in RESULTS. We calculated laten-
times and assumed that negative phase relations indicate
the entire range. We averaged phase angles over coherent seg-
ments. Finally, we subtracted each subject’s measured P-R interval from arterial pressure-R interval
latencies to derive true estimates of the latencies between
arterial pressure changes and sinoatrial node responses.

\[ G_{xy}(f) = (1 - \gamma_{x}^{2})G_{x}(f) \]

and

\[ \gamma_{x}^{2}(f) = \frac{|G_{xy}(f)|^2}{G_{x}(f)G_{y}(f)} \]

Bendat and Piersol (4) give further in-depth details about
these calculations.

The smoothed cross-spectra were determined by

\[ G_{xy}(f) = \int_{-\infty}^{\infty} w(u-f)X(u)Y(u)du \]

where \( M \) is the parameter determining the width of the
triangular smoothing window.

We averaged significant (\( \geq 0.50 \)) squared coherences and
partial coherences from time series cross-spectra within the
ranges 0.05–0.15 and 0.15–0.30 Hz. In subjects with multi-
ple significant coherence peaks, we arbitrarily chose coher-
ces and partial coherences that fell entirely within the
boundaries set. In subjects with continuous low-frequency
coherences and partial coherences that ended within the
0.05–0.15-Hz window but began at frequencies below 0.05
Hz, we averaged coherence and partial coherence over the
entire range. We averaged phase angles over coherent seg-
ments and assumed that negative phase relations indicate
that arterial pressure changes precede R-R interval changes
(we discuss this assumption in RESULTS). We calculated laten-
tices from the average phase angle and center frequency over
coherent segments. Finally, we subtracted each subject’s
measured P-R interval from arterial pressure-R interval
latencies to derive true estimates of the latencies between
arterial pressure changes and sinoatrial node responses.

Statistical analysis. Data are given as means ± SE. When
data were distributed normally, statistical comparisons
among variables were made with one-way ANOVA. When

\[ X(f) = \int_{-\infty}^{\infty} x(t)e^{i2\pi ft}dt \]

\[ Y(f) = \int_{-\infty}^{\infty} y(t)e^{i2\pi ft}dt \]

\[ w(f) = \frac{M - |f|}{2M}, \quad -M \leq f \leq M \]

or

\[ w(f) = 0 \] otherwise.

**RESULTS**

**Time domain analyses.** Average measurements from
the group of subjects were comparable during uncon-
trolled and fixed-frequency breathing, with one excep-
tion: end-tidal CO2 concentration was significantly
lower during fixed frequency than spontaneous breathing
(5.4 ± 0.1 vs. 4.7 ± 0.2%; \( P < 0.05 \)).

Figure 1 depicts measurements obtained from one
subject during hyperventilation (which began at time 0).
By about 30 s after the onset of hyperventilation,
R-R intervals, R-R interval fluctuations, systolic and
diastolic pressures, and (as expected) end-tidal CO2
concentrations had declined substantially, and muscle
sympathetic nerve activity had increased (in this sub-
ject, total nerve activity increased 40% during
hyperventilation). Figure 2 shows 15-s average mea-
surements during hyperventilation from all subjects.
The changes of the group of subjects were similar to those
shown for the individual subject (Fig. 1): R-R intervals
decreased and remained at a low level; systolic and
diastolic pressures fell transiently and then recovered;
and muscle sympathetic nerve activity increased dur-
ing the fall of arterial pressure and then drifted down-
toward baseline levels.

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Throughout this paper, we refer to “significant” (squared) coher-
ence as being \( \geq 0.50 \). This value, which indicates the strength of the
linear association between two spectra, was first proposed by de Boer,
Karemaker, and Strackee (9, 10) and was based on an appro-
priate mathematical analysis of their data. After this group’s semi-
nal publications, virtually all authors who use cross-spectral analy-
ysis have considered that squared coherence values \( \geq 0.50 \) indicate
significant relations. Although we have been critical of this nearly
universal usage (65), we use the value here, as a point of departure.
The results we report cast further doubt on determinations of sig-
nificance based on coherence, not on the basis of mathematics, but on
the basis of autonomic neurophysiology.

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Fig. 1. Experimental record from one subject during hyperventila-
tion.
Subjects were able to maintain apnea for an average of 290 s (range: 181–407 s). Figure 3 is a recording from one subject. In this and other subjects, R-R intervals increased dramatically at the onset of breath holding (from the low levels recorded at the end of hyperventilation) and then drifted downward toward baseline levels. R-R interval fluctuations were prominent during apnea. Arterial pressure (Fig. 3) fell initially and then rose as apnea continued. There was a major volley of muscle sympathetic nerve bursts at the onset of apnea (Fig. 3, left, bottom panel), concurrent with the abrupt reduction of arterial pressure, and a gradual increase of sympathetic activity as apnea continued. Sympathetic nerve activity was absent after the resumption of breathing (Fig. 3 left, bottom panel, extreme right).

During apnea, this and most other subjects had involuntary and unrecognized changes of abdominal girth. (We regard these as aborted breaths. However, in subjects in whom we recorded pneumotachograms, there was no air flow; therefore, we cannot exclude other possibilities, such as minor coughs or throat clearings.) One of these events (delimited by the vertical dashed lines in Fig. 3) is shown on an expanded scale on the right of Fig. 3. This change of abdominal girth was extremely small; the pressure gauge connected to the abdominal bellows was so sensitive that it registered pulsations of the abdominal aorta (Fig. 3, top right), and the Fleisch pneumotachograph recording (not shown) did not register any air flow. Nonetheless, the sequential hemodynamic changes set in motion by this small event were huge. R-R intervals fell from 1.19 to 0.94 s, and then rose to 1.40 s. Systolic pressures fell from 139 mmHg at the time of maximum R-R interval prolongation to 110 mmHg. During this pressure reduction, there was a major volley of eight large muscle sympathetic bursts in consecutive R-R intervals. During apnea in this and most other subjects, these changes occurred erratically. The autonomic and hemodynamic responses that followed (best seen in the R-R interval tracing, Fig. 3, second left panel), tracked the gasps, with identical periodicities.

Frequency domain analyses. Figure 4 depicts a fast Fourier transformation of muscle sympathetic burst amplitude spectral power from one subject during apnea. (For this analysis, spectra were calculated during 60-s windows moved by 1.5-s steps through the 180-s time series.) The contour map on the right comprises horizontal sections made from the three-dimensional representation on the left. (The highest peaks of spec-
tral power are shown in yellow.) This map clearly documents persistence of major fluctuations of muscle sympathetic nerve burst amplitude spectral power during apnea and indicates further that such sympathetic oscillations come and go at different low frequencies. Muscle sympathetic nerve activity during apnea was absent at respiratory frequencies.

Table 1 lists and Fig. 5 illustrates average measurements made during all breathing protocols. The significant differences among measurements are that R-R intervals were shorter during hyperventilation than during all other breathing protocols, and systolic and diastolic pressures were higher during apnea than during other breathing protocols. Muscle sympathetic nerve activity was significantly higher during apnea than during fixed-frequency breathing. End-tidal CO₂ levels, which had decreased significantly during fixed-frequency breathing, from those measured during spontaneous breathing, decreased further during hyperventilation.

Vagal baroreflex gain. We calculated the modulus of the transfer function between systolic pressure and R-R intervals (see METHODS) as 60-s windows moving by 1-s steps. Figure 6 illustrates such estimates of vagal baroreflex gain measured in two subjects during spontaneous breathing. We did not analyze these moving transfer functions exhaustively. However, even cursory inspection of our data indicated that transfer function moduli fluctuated quasiperiodically, in all subjects during all protocols (including apnea).

Other frequency domain analyses. Figure 7 depicts average (heavy lines) and individual (light lines) R-R intervals, systolic pressures, and muscle sympathetic nerve burst amplitude spectral powers for all breathing protocols. These results document substantial variability among spectral power measurements obtained from individual subjects and also among average measurements obtained during different protocols. R-R interval spectral power at respiratory frequencies (~0.25 Hz) was large during spontaneous and fixed-frequency breathing but nearly absent during hyperventilation and apnea (Fig. 7, right portions of top right two panels). Low-frequency R-R interval spectral power was present, but small, during spontaneous and fixed-fre-
Systolic pressure spectral power (Fig. 7, middle panels) was minimal at respiratory frequencies during all breathing protocols. However, there were major systolic pressure spectral peaks at breathing frequencies during hyperventilation (not shown: the breathing rate during hyperventilation averaged 0.44 Hz, or 26 breaths/min). Muscle sympathetic nerve burst amplitude spectral power (Fig. 7, bottom panels) was small during apnea breathing and hyperventilation and was large during apnea.

Systolic pressure spectral power (Fig. 7, middle panels) was minimal at respiratory frequencies during all breathing protocols. [However, there were major systolic pressure spectral peaks at breathing frequencies during hyperventilation (not shown: the breathing rate during hyperventilation averaged 0.44 Hz, or 26 breaths/min).] Muscle sympathetic nerve burst amplitude spectral power (Fig. 7, bottom panels) was small during apnea breathing and hyperventilation and was large during apnea.

### Table 1. Average measurements during the first and last minutes for all breathing protocols

<table>
<thead>
<tr>
<th>Protocol</th>
<th>R-R Interval, ms</th>
<th>Systolic Pressure, mmHg</th>
<th>Diastolic Pressure, mmHg</th>
<th>Sympathetic Nerve Activity</th>
<th>End-Tidal CO₂, %</th>
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<tr>
<td></td>
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<td></td>
<td></td>
<td>Bursts/min</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>Bursts/min × normalized burst area</td>
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<tr>
<td>Uncontrolled breathing</td>
<td></td>
<td></td>
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<tr>
<td>First min</td>
<td>924 ± 47&lt;sup&gt;a&lt;/sup&gt;</td>
<td>139 ± 9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>82 ± 5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>17 ± 3</td>
<td>16 ± 3&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
<td>Last min</td>
<td>924 ± 47&lt;sup&gt;a&lt;/sup&gt;</td>
<td>139 ± 8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>81 ± 5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>16 ± 2</td>
<td>15 ± 2&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
<td>Fixed frequency breathing</td>
<td>(0.25 Hz)</td>
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<tr>
<td>First min</td>
<td>934 ± 48&lt;sup&gt;a&lt;/sup&gt;</td>
<td>138 ± 7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>78 ± 3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>15 ± 3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>17 ± 3&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
<td>Last min</td>
<td>937 ± 44&lt;sup&gt;a&lt;/sup&gt;</td>
<td>140 ± 6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>79 ± 3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>13 ± 3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>12 ± 2&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>Hyperventilation</td>
<td></td>
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<tr>
<td>First min</td>
<td>676 ± 32&lt;sup&gt;b&lt;/sup&gt;</td>
<td>131 ± 7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>76 ± 2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>22 ± 4</td>
<td>26 ± 4&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
<td>Last min</td>
<td>652 ± 32&lt;sup&gt;b&lt;/sup&gt;</td>
<td>143 ± 9&lt;sup&gt;c&lt;/sup&gt;</td>
<td>82 ± 4&lt;sup&gt;c&lt;/sup&gt;</td>
<td>20 ± 3</td>
<td>22 ± 3&lt;sup&gt;c&lt;/sup&gt;</td>
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<tr>
<td>Apnea</td>
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<tr>
<td>First min</td>
<td>978 ± 48&lt;sup&gt;a&lt;/sup&gt;</td>
<td>147 ± 8&lt;sup&gt;b&lt;/sup&gt;</td>
<td>89 ± 4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>20 ± 3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>26 ± 4&lt;sup&gt;b&lt;/sup&gt;</td>
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<tr>
<td>Last min</td>
<td>927 ± 48&lt;sup&gt;a&lt;/sup&gt;</td>
<td>166 ± 10&lt;sup&gt;b&lt;/sup&gt;</td>
<td>103 ± 5&lt;sup&gt;c&lt;/sup&gt;</td>
<td>24 ± 4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>33 ± 5&lt;sup&gt;b&lt;/sup&gt;</td>
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</table>

Data are average measurements (±SE) for all subjects. Letters and symbols denote statistical significance. Values in each column without a letter or values that share the same letter are not significantly different from each other. Values within each column with different letters are significantly different from each other. For example, the average systolic pressure during the last min of apnea was 166 ± 10<sup>b</sup>. b indicates that this pressure was significantly higher than the average pressure during any other breathing protocol. † indicates that the pressure during the last min of apnea was also significantly higher than the average pressure during the first min of apnea.

**Average measurements: all subjects, all protocols (n = 9)**

![平均测量](http://ajpheart.physiology.org/)
at respiratory frequencies, but was large over a wide range of low frequencies during all protocols. Statistical analysis of the integrated low-frequency spectral powers depicted in Fig. 7 failed to identify any significant differences among measurements during the different breathing conditions and apnea.

Figure 8 depicts sliding squared coherence and coherence after partialization (see METHODS) of the relations between systolic pressures and R-R intervals made during 5 min of spontaneous breathing in one subject. For these analyses, the window for each spectrum was 90 s, and the steps were 3 s. (Thus the first calculation was made 45 s into the breathing epoch, and the last was made 45 s before its conclusion.) The horizontal dashed lines in coherence and partial coherence analyses (Fig. 8, left) indicate the level, 0.50 (10, 65), which as mentioned, is used almost universally to reflect significant coherence in studies of human autonomic oscillations.

The top left of Fig. 8 indicates that in this subject, systolic pressures and R-R intervals were broadly coherent over low (0.052–0.092) and respiratory frequency (0.19–0.33 Hz) ranges. The top right of Fig. 8 indicates that the average phase angles over coherent frequencies were $-50^\circ$ at the low frequency, and $-32^\circ$ at the respiratory frequency. If systolic pressures lead R-R intervals in such cross-spectral analyses (see discussion below), these phase angles translate into latencies (to the P wave) of 1.73 and 0.15 s at low and respiratory frequencies.

The results of partialization (with respiration as the first input signal, the one whose influence was removed, and systolic pressure as the second input signal) are shown in Fig. 8, bottom. Significant coherence persisted after partialization at low frequencies (0.048–0.084 Hz), with an average phase angle of $-52^\circ$.
(latency: 2.00 s). Removal of respiratory influences by partialization abolished the coherence and systematic phase angle between systolic pressure and R-R interval cross spectra at respiratory frequencies (Fig. 8, right portions of bottom panels).

Figure 9 shows average coherence and coherence after removal of respiratory influences by partialization and corresponding phase angles for all subjects. These results document for the group what was apparent for the individual subject (Fig. 8). For the group, average coherence between systolic pressure and R-R interval time series at respiratory frequencies was strikingly high (peak: 0.93, Fig. 9, top right, light line). Average coherence at respiratory frequencies fell to below 0.50 after partialization (Fig. 9, top right, heavy line).

For the group of subjects, latencies between systolic pressure and P wave fluctuations averaged $-1.62 \pm 0.20$ at low frequencies and $-0.03 \pm 0.12$ s at respiratory frequencies. All latencies at low frequencies were negative; five of nine latencies at respiratory frequencies were positive. If, as we assume, negative latencies indicate that systolic pressure changes precede P-P interval changes (the mathematical computation does not indicate which signal precedes the other), then positive latencies at respiratory frequencies indicate that systolic pressure changes follow P-P interval changes. Latencies at low frequencies were significantly ($P < 0.001$) longer than latencies at respiratory frequencies. There was no significant difference between low-frequency latencies calculated before and after partialization ($-1.62 \pm 0.20$ vs. $-1.46 \pm 0.28$, $P = 0.33$, Mann-Whitney rank sum test).

Figure 10, top two panels, shows average (heavy lines) and individual (light lines) coherence between respiration and diastolic pressure and muscle sympathetic nerve burst amplitude cross spectra during fixed-frequency breathing. These analyses support conclusions that were not expected and that might even be counterintuitive. First, over a wide range of frequencies, from the low to the respiratory frequency, the average coherences between respiration and diastolic pressure and muscle sympathetic nerve burst amplitude spectra did not exceed the accepted threshold level for significance, 0.50 (horizontal line). Even at the breathing frequency (set at 0.25 Hz), average coherence barely exceeded 0.50. Second, across subjects there is great variability of coherent frequencies below the respiratory frequency. Third, partialization (Fig. 10, bottom panel) reduced average coherences between diastolic pressure and R-R interval and muscle sympathetic nerve burst amplitude spectra at the breathing frequency to below 0.50.

**DISCUSSION**

We performed a simple study: we asked nine healthy young adult volunteers to breathe in different ways (or to not breathe at all) while we measured their R-R
intervals, arterial pressures, and muscle sympathetic nerve activity. We analyzed their responses mathematically and drew inferences that may have important implications regarding how human autonomic neural organization should be viewed.

First, breathing at usual frequencies does not merely entrain autonomic rhythms; respiratory activity is a necessary condition for such oscillations to occur. Second, the very strong coherence that may exist among arterial pressure, R-R interval, and muscle sympathetic nerve oscillations at breathing frequencies results from respiratory influences on these variables; in healthy supine human subjects, this temporal association does not reflect arterial baroreflex physiology. Third, respiration at usual frequencies exerts no influence on low-frequency autonomic rhythms; low-frequency rhythms are not altered by differences of breathing frequency and persist undiminished in the absence of breathing. Finally, and most importantly, the coherence and coherent frequencies among autonomic and hemodynamic oscillations vary continuously and profoundly. Therefore, the dimension (time) must be considered in analyses of human autonomic interrelations; the constructs of “stationarity,” “steady-state,” and even “significant coherence” may have little relevance.

Autonomic and hemodynamic responses to different breathing modes and apnea. The low-frequency fluctuations we recorded were not influenced significantly by hyperventilation. Barman and Gebber (2) reported that in most of the anesthetized cats they studied, low-frequency carotid postganglionic sympathetic rhythms persisted after phrenic nerve activity had been silenced by hyperventilation. They took their observations (and we take ours) to mean that low-frequency sympathetic rhythms are generated by mechanisms that are independent of respiratory rhythm generators. Our results contradict the observation of Hyndman et al. (34) that rapid breathing abolishes low-frequency arterial pressure waves.

Apnea. As ably reviewed by Koepchen (39), the arterial pressure waves described by Traube (67) and E. Hering (33) occurred at respiratory frequencies. However, both Traube and Hering also reported persistence of slower arterial pressure waves during apnea. We documented persistence of low-frequency arterial pressure, R-R interval, and muscle sympathetic nerve fluctuations during apnea (Figs. 4 and 7). The subjects we studied maintained apnea for long enough periods (181 to 407 s) to allow us to state with confidence that their low-frequency rhythms persisted. Our observation that low-frequency arterial pressure rhythms persist during apnea confirms results published earlier by Dornhorst et al. (13), Joels and Samueloff (35), and Passino et al. (52). Our finding that muscle sympathetic nerve rhythms also persist at low frequencies during apnea confirms results published preliminarily by Pawelczyk and Levine (53).

We did not observe persistence of respiratory-frequency R-R interval fluctuations during apnea (Fig. 7), as reported by Trzebski (68) and Piepoli (56) and their co-workers. However, we did observe that what we took to be aborted breaths, triggered very large hemodynamic responses during apnea (one is shown in Fig. 3, right). Our subjects’ responses to those events (whatever they were) were similar to those described by Peňáz and Buriánek (54), who recorded responses to single breaths, introduced deliberately during apnea. Katona et al. (36) reported that attempts of anesthetized but spontaneously breathing dogs to breathe after their respiratory drive had been suppressed by hyperventilation provoked abrupt reductions of vagal-cardiac nerve traffic. We speculate that the major abrupt autonomic and hemodynamic changes that occurred with these apparent aborted breaths during apnea were caused by respiratory motoneuron activity. If we are correct, these oscillations probably were not
caused by afferent inputs from lung and thoracic stretch receptors, because no air movement (as gauged by a Fleisch pneumotachograph) occurred. [This inference contradicts a conclusion reached by St. Croix and colleagues (63) that respiratory motoneuron activity makes a small or no contribution to respiratory modulation of muscle sympathetic nerve activity.] It also is unlikely that these oscillations represent responses to some intrinsic pacemaker; they were time locked to the erratic, small pneumograph signals that punctuated apnea.

Muscle sympathetic nerve activity increased during apnea compared with levels measured during fixed-frequency breathing. An earlier study documented significant, steady increases of muscle sympathetic nerve activity during very brief periods of apnea (23). Because arterial pressure also rose during apnea (Fig. 5), such parallel changes indicate that apnea resets the usual relation that exists between arterial pressure and muscle sympathetic nerve activity (45). We did not study mechanisms responsible for apneic increases of sympathetic activity and arterial pressure. However, others (30, 47) have implicated small reductions of sympathetic and arterial pressure. However, published literature indicates clearly that a reflex, resonant mechanism is not necessary to explain low-frequency arterial pressure waves. Low-frequency arterial pressure waves persist when pressure in arterial baroreceptive arteries is held constant (26), and when baroreflex participation is precluded by sinoaortic denervation (22, 42).

Thus some published studies suggest that low-frequency arterial pressure waves are driven importantly by central oscillations. Accordingly, we had expected to find strong coherence between low-frequency muscle sympathetic nerve and arterial pressure rhythms. However, although coherence between diastolic pressure and muscle sympathetic burst amplitude spectra was highly significant in individual subjects, coherence over low frequencies [defined according to conventional usage (10, 65) as \( r = 0.50 \)] was insignificant for the group at large. We discuss this unexpected negative observation below.

Respiratory frequency rhythms. We documented strong coherence between systolic pressure and R-R interval spectra at breathing frequencies (Figs. 8 and 9). Vagal baroreflex gain, as estimated by moduli of transfer functions at breathing frequencies, may be quantitatively similar to baroreflex gain estimated at low frequencies and to baroreflex gain measured after bolus phenylephrine injections (50, 73). This gain is diminished greatly by sinoaortic baroreceptor denervation. Therefore, it is not surprising that de Boer (11) and Pagani (3, 50) and their associates suggested that consensual changes of systolic pressure and R-R intervals at respiratory frequencies reflect baroreflex physiology: breathing modulates arterial pressure, which in turn, modulates R-R intervals. Several of our results and results published by others provide what we consider compelling evidence against this hypothesis.

As mentioned, despite the advantages human subjects bring to physiological research, they also bring major disadvantages. Perhaps none is more important than the fact that human subjects are studied with their reflex loops “closed.” Because of this, statistically significant associations between time series do not necessarily prove causality. Indeed, it is difficult to know from these associations what is cause and what is effect, or even if there is a cause-and-effect relation at all. We approached this challenge in a new way by analyzing signals with partial coherence. Partial coherence analysis is a technique (4, 43) that mathematically removes the influences of one signal (in our case, respiration) on two other signals (systolic pressure and R-R intervals) and then determines coherence between the residual components of the other two signals. Our results suggest strongly that correspondence between systolic pressures and R-R intervals at respiratory frequencies is secondary to the respiratory influence these signals share. This point is made graphically by
the analyses from one subject depicted in Fig. 8 and from the analyses of group responses depicted in Fig. 9.

Our measurements of phase angle (the more apposite measure is latency) provide additional evidence against a baroreflex explanation for the strong coherence between systolic pressure and R-R interval signals at breathing frequencies. As discussed, latencies at low frequencies were always negative, were quite constant, and were such that they could be explained simply by vagal baroreflex physiology. Latencies at breathing frequencies, however, were positive more often than they were negative, were quite variable among subjects, and were too short to be explained by vagal baroreflex physiology.

Vagal baroreceptor-cardiac reflex latency is the pure time delay between the beginning of a baroreceptor stimulus and the earliest P-P interval prolongation it provokes (20). An earlier study (14) broke out the individual components that are lumped together as “baroreflex latency.” This analysis suggested that most (about two-thirds) of the short vagal baroreceptor-cardiac reflex latency in humans results from delays in the influence of acetylcholine on sinoatrial node depolarization. The average latency between respiratory frequency systolic pressure and P-P interval fluctuations in our study was ~0.03 s. This value is substantially shorter than the shortest baroreflex latency published from human studies (0.24 s; see Ref. 14). Moreover, if vagal baroreflex latencies reflect primarily delays in the action of acetylcholine on sinoatrial pacemaker cells, it is unclear why latencies at respiratory frequencies should be so variable among subjects.

Another important question is, Why are systolic pressure-R-R interval latencies much shorter at respiratory than low frequencies? One explanation is that latencies at respiratory frequencies reflect rapid vagal responses, whereas latencies at low frequencies reflect some admixture of sluggish sympathetic and rapid vagal responses. This explanation seems unlikely, because removal of potential sympathetic contributions by β-adrenergic blockade does not alter systolic pressure-R-R interval latencies at low frequencies (27).

We suggest that the most parsimonious answer to the questions posed above is that the close correlation between systolic pressures and R-R intervals at breathing frequencies reflects the influence of respiration on arterial pressure and R-R interval rhythm generators and not baroreflex physiology. This interpretation can explain why when subjects breathe at different rates, the latency between their breaths and R-R intervals is constant, but the latency between their arterial pressures and R-R intervals is highly variable (44).

In this connection, reductions of the respiratory frequency moduli of transfer functions by baroreceptor denervation do not necessarily prove that respiratory frequency R-R interval changes reflect baroreceptor physiology. Vagal-cardiac motoneurons fire primarily because they are stimulated by arterial baroreceptors (62). Schweitzer (59) asserted that two influences are necessary for the generation of respiratory sinus arrhythmia: 1) integrity of brain stem respiratory centers, and 2) baroreceptor stimulation of vagal-cardiac motoneurons. In this context, great reductions of respiratory frequency R-R interval fluctuations can be explained economically, on the basis that surgical denervation (or pharmacological hypotension (19)) reduces the baroreceptor stimulation that is necessary for vagal-cardiac motoneuron firing to occur and then to be modulated by breathing (24).

Our results also suggest that some respiratory frequency variations of muscle sympathetic nerve activity reflect the influence of respiration rather than baroreflex physiology. Partialization of diastolic pressure and muscle sympathetic burst amplitude spectra substantially reduced the squared coherence that was found with simple coherence analysis (Fig. 10, bottom panel, right). These results confirm in a new way results published by Adrian et al. (1), who showed that respiratory grouping of sympathetic nerve activity persists in anesthetized cats after sinoaortic denervation. Moreover, they can be explained by an earlier study (18), which showed that respiration “gates” responsiveness of muscle sympathetic motoneurons to baroreceptor influences, just as it does responsiveness of vagal-cardiac motoneurons (17, 24, 32).

Coordination among human autonomic and hemodynamic rhythms. The foregoing discussion implicitly poses the question, Are the ongoing autonomic and hemodynamic fluctuations observable in healthy resting humans caused by intrinsic oscillators or by reflex mechanisms? We propose that this question, which is fundamental to the physiology we studied, is not one that admits of easy answers. Moreover, it may not even be a good question.

If any feature of our measurements stands out, it is their lack of stationarity. We documented major transients of autonomic function during hyperventilation (Figs. 1 and 2), apnea (Fig. 4), and even fixed-frequency breathing (Fig. 6), which we took to be the most “steady-state” condition we studied. Several authors (25, 34, 74) have wrestled with the question, If human pressure-regulating reflexes are intact, why does arterial pressure vary?; and they have proposed that pressure varies because arterial baroreflex gain varies. Earlier studies have documented augmentation of vagal baroreflex gain during sleep (61) and unexplained fluctuations of baroreflex gain during waking hours (51). Our study identifies ongoing quasiperiodic fluctuations of baroreflex gain in supine, resting, unper-turbed human subjects (two examples are shown in Fig. 6). Such fluctuations were present in varying degrees in all our subjects during all breathing protocols and apnea.

We were surprised by the general lack of coherence between respiration and diastolic pressure and muscle sympathetic nerve activity (Fig. 10). [However, some animal studies (6, 55) also failed to identify significant coherence between sympathetic nerve activity and arterial pressure.] A wealth of earlier research documents strong correlations between sympathetic nerve activity and breathing (6, 55) and diastolic pressure.
We considered that if we could explain this apparently paradoxical result, we might be on the way to explaining the poor coherence among the signals we recorded. Figure 11 may have heuristic value in explaining variabilities in human autonomic signals.

For these analyses, we selected data from two subjects, one with strong, and the other with weak coherence among respiration and diastolic pressure and muscle sympathetic nerve activity. The top panels of Fig. 11 are contour maps derived from sliding (60-s windows, moved in 2-s steps through 180 s) fast Fourier transformations of respiration. These analyses indicate that during these periods of fixed-frequency breathing, variability of autonomic signals cannot be ascribed to variability of respiration. The middle panels of Fig. 11 depict average diastolic pressure and muscle sympathetic burst amplitude squared coherence measured during the same breathing epochs. The subject on the left had strong coherence between diastolic pressure and sympathetic nerve activity at low and respiratory frequencies, and the subject on the right had only borderline coherence at respiratory frequencies.

The bottom panels of Fig. 11 are contour maps of sliding squared coherence. The contour maps show only those times and frequencies with coherences ≥0.50. [In this scheme, coherences ≤0.50 are white, coherences ≥0.50 are colored, and the highest levels of coherence (~0.80 -1.0) are yellow.] These analyses indicate that coherence between diastolic pressure and muscle sympathetic nerve activity varies not only over time but also over frequency. They also indicate, counterintuitively, that both subjects, including the one on the right with marginally significant average coherence, had episodic, highly significant levels of coherence.

The subject on the left panel of Fig. 11 had “significant” average coherence (left middle panel), because his strong coherence persisted over low and respiratory frequency ranges during most of the recording period. Conversely, the subject on the right panel had weak coherence, not because his diastolic pressure and muscle sympathetic nerve activity were uncoordinated, but because his strong coherence came and went and shifted over a range of frequencies. One shift extends from slightly below 0.15 at the beginning of the breathing period (Fig. 11, bottom of the bottom right panel) to almost 0.30 Hz at the end of the breathing period. Thus, in resting humans, autonomic coordination is not qualitative, present or absent, significant or insignificant, or above or below squared coherence values of 0.50, but is quantitative and is based on the probability that strong coherence will persist over time at more or less constant frequencies.

The notion that coordination between centrally generated neural outflows is sliding, not fixed, was ad-
vanced first by von Holst (69) on the basis of his studies of the fin movements of fish and was championed over many years by Hans-Peter Koepchen (38) on the basis of his research in animals. Most studies reporting transitions of autonomic rhythms deal with anesthetized animals in which shifts of autonomic coordination occur after defined, sometimes major changes, including panting (41), voluntary body movement (48), and cerebral ischemia (37). Our study extends this literature by showing that sliding coordination is an ongoing feature of autonomic physiology, and that it can be observed in resting humans under very stable circumstances with no discernible provocation whatever.

The principal limitation of our study is the brief duration of the recording periods. Because of our limited periods of observation, we were unable to determine whether the autonomic fluctuations we recorded are stochastic or probabilistic, periodic or aperiodic, or even cause and effect. Second, measurements we obtained during apnea must not be considered to reflect usual human physiology; we used apnea following hyperventilation with 100% oxygen as one more experimental tool to remove respiration as a variable. The measurements we obtained during apnea illustrate a third potential limitation: the occurrence of what we regard as aborted breaths. These events occurred in almost all of our subjects and were not recognized at the time by either the subjects themselves or by the team making the recordings. A fourth (and major) limitation is that human sympathetic bursts occur intermittently, not continuously. We attempted to deal with this inherent limitation by averaging muscle sympathetic nerve activity over 1-s intervals and performing analyses on these equidistant measurements. Finally, all of the results of our study are based on linear analyses; therefore, our conclusions may not apply to situations in which relations vary nonlinearly, as they surely must in resting humans.

In conclusion, our study treats relations among autonomic and hemodynamic variables in a group of healthy young adults, as affected by different breathing modes and apnea. Although we show that in supine resting humans, breathing exerts no measurable influence on low-frequency rhythms, we also show that that breathing is prepotent in generating respiratory frequency rhythms. Arguably, the most important contribution our study makes is to underscore the fluidity of human autonomic transactions. Our conclusions are fully compatible with Koepchen’s summary (38) of autonomic periodicities: “Their interactions are not unidirectional but bidirectional. Not fixed but sliding, not obligatory but facultative and comprise not only one but several rhythms.”

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